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* *From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1–3, 1985).

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5-FU Infusion in Advanced Colorectal Cancer: A Comparison of Three Dose Schedules[1]

Amil Shah,[2,3,*] Walter MacDonald,[2,3] James Goldie,[2,3] Gedy Gudauskas,[3] and Barbara Brisebois[3]

Ninety-four patients with advanced colorectal adenocarcinoma were treated by continuous iv 5-FU infusion on three different dose schedules. Thirty-three patients received a 72-hour infusion of 5-FU (30 mg/kg/24 hours) every 3 weeks (Group A); 31 received a 72-hour infusion of 5-FU (30 mg/kg/24 hours) every 2 weeks (Group B); and 30 received a 48-hour infusion of 5-FU (30 mg/kg/24 hours) every week (Group C). Although this was a sequential nonrandomized study of the dose schedules, the groups were comparable with respect to various prognostic factors. Response rates were as follows: *Group A*—three patients had minor response (9%) and 30 had no response (91%); *Group B*—five patients achieved partial response (16%), nine had minor response (29%), and 17 had no response (55%), and *Group C*—one patient achieved complete response (3%), eight achieved partial response (27%), five had minor response (17%), and 16 had no response (53%). The median survival time for Group A was 9 months, for Group B was 9.5 months, and for Group C was 14 months. Intensifying the dose schedule of 5-FU by increasing the frequency of administration has significantly improved response rates. A prolongation of the median survival time of patients treated with a 48-hour infusion at 1-week intervals was noted, although this was not statistically significant. [Cancer Treat Rep 69:739–742, 1985]

The chemotherapeutic management of advanced colorectal cancer is disappointing. 5-FU has been extensively studied, but there is no consensus as to its optimal dose schedule (1). In our study, the use of weekly bolus injection of 5-FU (15 mg/kg) has produced a partial response rate of 4%. Further, combination chemotherapy in colorectal cancer has not been consistently demonstrated to be superior to single-agent treatment with 5-FU (2). These facts prompted us to explore different dose schedules of 5-FU in an attempt to improve response rates without excessive toxicity. We report our experience in the treatment of advanced colorectal adenocarcinoma with iv 5-FU infusion given by three different continuous-infusion schedules: 72 hours every 3 weeks; 72 hours every 2 weeks; and 48 hours weekly.

MATERIALS AND METHODS

This series of studies extended over the period from October 1979 to March 1982. Patients referred to the Cancer Control Agency of British Columbia with unresectable metastatic colorectal cancer were carefully reviewed and the disease extent documented before entry to this protocol. All patients had histologic proof of colorectal adenocarcinoma, a site of measurable disease, and a life expectancy of > 8 weeks. Patients were not excluded because of previous treatment with weekly 5-FU bolus injections. Informed consent was obtained. This was a nonrandomized, sequential study of three dose schedules of 5-FU infusion.

Table 1 shows the treatment schedules of chemotherapy. The dose of 5-FU was increased with increasing frequency of administration. Thus, patients received 180 mg/kg over a 6-week period in Schedule A, 270 mg/kg in Schedule B, and 360 mg/kg in Schedule C. From 1979 to 1980, 5-FU was administered on an inpatient basis as a continuous iv infusion in 5% dextrose in water by the gravity-drip method. From 1981 to 1982, most patients were treated on an outpatient basis using the Ambulatory CorMed pump Model ML6-4.

[1]Received Feb 17, 1984; revised Oct 24, 1984; accepted Dec 8, 1984.

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TABLE 1.—Treatment schedules of 5-FU infusion

Group	5-FU dose (mg/kg/24 hrs)	Duration of infusion (hrs)	Frequency of infusion	Dose rate (mg/kg/wk)
A	30	72	Every 3 wks	30
B	30	72	Every 2 wks	45
C	30	48	Weekly	60

Complete response (CR) was defined as the disappearance of all detectable disease for at least 8 weeks. Partial response (PR) was defined as a > 50% decrease in the products of the perpendicular diameters of all measurable disease for at least 8 weeks. PR of malignant hepatomegaly was defined as a > 50% decrease in the sum of the liver measurements below the costal margins in the midclavicular lines and the tip of the xiphoid. Minor response was defined as a < 50% decrease in the tumor areas for at least 8 weeks or a < 50% decrease in the sum of the liver measurements for malignant hepatomegaly. No change or any increase in the size of the tumor dimensions or the appearance of a new site of disease was defined as no response. Treatment was considered adequate for evaluation if the patient received one cycle of 5-FU infusion.

Patients were evaluated clinically and by appropriate imaging techniques after three cycles of treatment. Those patients who had a favorable response continued receiving treatment until achievement of CR or documentation of progressive disease.

The durations of response and survival are calculated from the start of 5-FU infusional chemotherapy. Tests for differences in response rates were performed using the Kaplan-Meier method (3), and differences in survival times are assessed by the test described by Mantel (4).

RESULTS

Patient Characteristics

Table 2 shows the major pretreatment characteristics of the patients. Ninety-four patients were entered in this series: 33 in Group A; 31 in Group B; and 30 in Group C. All patients had adequate treatment and all were evaluated. The three patient groups were comparable with regard to age, sex, and Eastern Cooperative Oncology Group (ECOG) performance status. There was no significant difference in the distribution of the site of dominant involvement by malignancy. Thirty-five patients were previously treated with weekly 5-FU bolus injection (15 mg/kg) for 3–5 months: ten in Group A; 14 in Group B; and 11 in Group C. All of these patients had evidence of disease progression before starting 5-FU infusional therapy. No patient was irradiated within 6 months of the start of chemotherapy. The histologic grades of the malignancy were comparable. In summary, although this was a sequential study of the three treatment schedules, the patients in the three groups appeared to be comparable with regard to those prognostic factors that were evaluated.

Toxicity

Toxicity was generally mild and included stomatitis in three patients in Group A and in three in Group B. This was avoided in the subsequent treatment of these patients by infusing the 5-FU at the same rate, but over a shorter period of 60 hours. Stomatitis was not experienced by any Group C patients. Four patients developed a diffuse macular erythematous rash over the trunk that was exacerbated during the 5-FU infusion. Six patients in Group C experienced a burning sensation in the palms and soles followed by erythema and hyperkeratosis of these areas. The dermatologic toxicity was minor and did not require any modification of the dose schedule of 5-FU. Gastrointestinal side effects were uncommon. Three patients had mild nausea. Neither vomiting nor diarrhea was observed. Two patients, one each in Groups B and C, experienced ataxia after 14 and 16 months of treatment. This complication was disabling in one patient and necessitated the discontinuation of treatment. The second patient was removed from the treatment because of disease progression. No case of seizures, motor paralysis, or sensory deficit was noted. Two patients in Group C had retrosternal discomfort during the 5-FU infusion. There was no antecedent cardiac history and electrocardiogram and cardiac enzymes monitoring during the chemotherapy infusion were normal. Chemotherapy was temporarily discontinued in one of these patients, but resumed when her disease progressed. The patient died 2 months

TABLE 2.—Pretreatment patient characteristics*

	Group—		
	A	B	C
Total	33	31	30
Male/female	19/14	16/15	19/11
Age (yrs)			
Median	58	62	59
Range	38–78	28–74	24–73
ECOG performance status			
0	3	2	3
1	14	19	16
2	12	9	8
3	4	1	3
Site of dominant metastatic disease			
Local abdominal /pelvic mass	3	3	5
Liver	20	18	19
Peritoneum	2	2	2
Lung	8	8	4
Histologic grade of malignancy			
Well or moderately well-differentiated	30	27	25
Poorly differentiated	3	4	5
Prior 5-FU bolus chemotherapy	10	14	11

* Unless otherwise specified, values = No. of patients.

later of progressive malignant disease. The other patient continued on treatment for 5 months until documentation of disease progression. Chemical phlebitis along the course of the vein used for 5-FU infusion was a troublesome problem in 15 patients who were treated with the CorMed pump. An arteriovenous Teflon loop graft between the femoral artery and greater saphenous vein was implanted for drug delivery with satisfactory results. No patient experienced bone marrow suppression or alopecia. Two of the patients in Group C with extensive peritoneal disease to which loops of small bowel were adherent developed an acute abdomen after 4 and 7 weeks of treatment, respectively. At laparotomy, the adherent loops of small bowel were edematous, with areas of necrosis and perforation. Both patients died of uncontrolled sepsis shortly after laparotomy.

Response Rates and Survival

Table 3 shows the response rates. In Group A patients, there were no PRs or CRs. Three patients (9%) had minor response lasting 2, 5, and 8 months, respectively. In Group B patients, five (16%) achieved PR of 5 months' median duration (range, 4-20 months), and nine (29%) had minor response of 3.5 months' median duration (range, 3-6 months). In Group C patients, one (3%) achieved CR lasting 26 months; eight (27%) achieved PR of 9 months' median duration (range, 5-17 months); and five (17%) had minor response of 5.5 months' median duration (range, 2.5-12 months). Comparison of the CR and PR rates of the three schedules was statistically significant ($P = 0.004$).

The response rates (PR + CR) for metastatic disease sites were the following. In Group B, two of three patients (67%) with abdominal or pelvic mass responded; one of 18 (6%) with liver metastasis responded; none of two (0%) with peritoneal metastasis responded; and two of eight (25%) with lung metastasis responded. In Group C, none of five patients (0%) with abdominal or pelvic mass responded; seven of 19 (37%) with liver metastasis responded; none of two (0%) with peritoneal metastasis responded; and two of four (50%) with lung metastasis responded. When responders (PR + CR) from all treatment groups were analyzed together and compared to those with minor response or no response, there was no significant difference in age, sex, initial performance status, prior chemotherapy, or grade of malignancy.

TABLE 3.—Response*

	Group—		
	A ($n = 33$)	B ($n = 31$)	C ($n = 30$)
CR	0	0	1 (3)
PR	0	5 (16)	8 (27)
Minor response	3 (9)	9 (29)	5 (17)
No response	30 (91)	17 (55)	16 (53)

* Values = No. of patients (%).

The median survival time for Group A was 9 months (range, 2-46); for Group B was 9.5 months (range, 4-31); and for Group C was 14 months (range, 1-32+) (fig 1). The overall survival of Group C was prolonged compared with that of either Group A or B, but this was not statistically significant ($P = 0.09$).

DISCUSSION

5-FU is the most extensively studied drug in the treatment of advanced colorectal cancer and is considered the "standard" treatment. There is much confusion regarding the optimal dose scheduling of 5-FU. The most popular

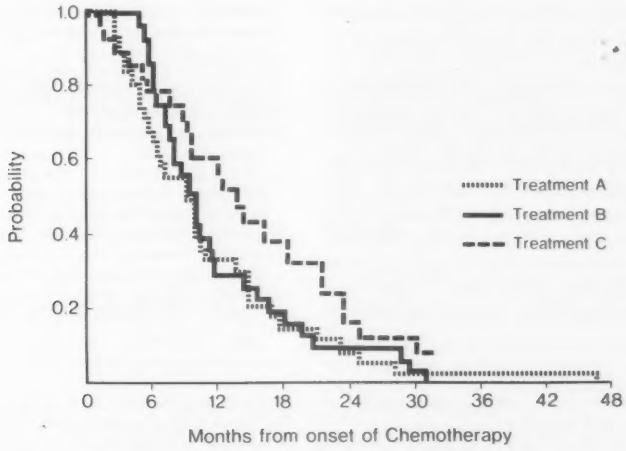

FIGURE 1.—Survival of patients tested with 5-FU infusion.

method of administration is by iv bolus injection, either by the "loading-dose" schedule of daily injections for 5 consecutive days followed by half doses on alternate days until toxicity or as weekly bolus injections (5–8). Our experience with the weekly iv bolus of 5-FU (15 mg/kg) has been disappointing, with two PRs (4%) and two minor responses (4%) observed in 50 consecutively treated patients. The loading-dose schedule is reported to give a better response rate, but the toxicity is greater (6). A few investigators have reported improved response rates when 5-FU is given as a continuous iv infusion (9,10). Seifert et al (9) reported that the dose-limiting side effect of the 5-FU infusional therapy was stomatitis, and this appeared to be related to the dose and duration of the infusion. Our initial experience with 120- and 96-hour infusions at 30 mg/kg/24 hours repeated every 4–5 weeks confirmed this observation; there was unacceptable toxicity if the full dose was used. We subsequently examined the efficacy of a 72-hour infusion every 3 weeks (Group A), which, over a given period, provides an average dose similar to that of the 96-hour infusion every 4 weeks or the 120-hour infusion every 5 weeks. The results of this study were disappointing, with a 9% minor response rate observed in a group of 33 patients. The schedule was, however, free of serious stomatitis or myelosuppression. In the second series of 31 patients, the treatment interval was reduced to 2 weeks (Group B). Toxicity was again mild. An improvement in the response rate was achieved, with a PR rate of 16%. The median survival time of Group B patients was not significantly extended in comparison with that of Group A patients. In the third series of 30 patients, a further intensification of the dose schedule was carried out with the weekly administration of a 48-hour 5-FU infusion (Group C). Side effects were more frequent, but still quite mild. There was no myelosuppression or stomatitis. A disturbing observation was the occurrence of small bowel perforation in two patients in whom there was extensive peritoneal spread of malignant disease to which loops of small bowel were adherent and likely infiltrated by malignancy. Perforation of involved bowel occasionally occurs unrelated to chemotherapy. It is uncertain whether small bowel perforation of two of 30 patients was due to chance or related to chemotherapy. With the intensive dose scheduling of Group C, a further improvement of response rates was achieved, with one patient (3%) achieving CR and eight (27%) achieving PR. The median survival time of 14 months for Group C patients was prolonged in comparison with that of either the 3- or 2-week schedules, but this was not statistically significant.

Although this was a sequential nonrandomized series, the treatment groups were comparable with respect to such prognostic factors as age, sex, performance status, site of metastatic disease, histologic grade of malignancy, and prior chemotherapy treatment. This study suggests an important schedule dependency in the action of 5-FU infusional therapy. By increasing the frequency of treatment cycles, and thus the dose, higher response rates were achieved with the weekly administration of a 48-hour infusion. There was no myelosuppression or stomatitis, and other side effects were mild. We think that this regimen dissociates response from toxicity to a greater degree than the weekly iv push, iv loading dose, and the 120-hour infusion schedules.

The results seem at least as good as the more prolonged and inconvenient continuous infusion (10). Because one of the purposes of our study was to minimize toxicity without decreasing the response rates, the doses of each schedule were not escalated to maximal tolerance. It is clear that further studies are needed to determine the optimal dose schedule of 5-FU. We are now conducting a study of dose escalations of 5-FU infusion at 1-week intervals to examine the effect of the total 5-FU dose on response rates and how response relates to toxicity.

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Prospectively Randomized Toxicity Study of High-Dose Versus Low-Dose Treatment Strategies for Lymphoblastoid Interferon[1,2]

Hulbert K. B. Silver,* Joseph M. Connors, and Fernando A. Salinas[3,4]

It is unclear from preliminary laboratory studies whether a high- or a low-dose interferon treatment strategy is optimal. As part of an ongoing study of mechanisms of interferon action, we have evaluated toxicity in a two-arm protocol in which patients were randomly assigned to receive lymphoblastoid interferon by either a low-dose treatment strategy (2×10^6 units/m^2 daily × 28 days then daily × 5 days every other week by im injection) or a high-dose treatment strategy (5×10^6 units/m^2 by continuous iv infusion over 24 hours, escalating by 5×10^6 units/m^2/day as tolerated over 10 days, repeated every 28 days). The main toxic effects in both arms were fever, fatigue, and anorexia. Marked interpatient differences within each dose arm were greater than differences between arms. Additional significant toxic effects included nausea and vomiting, hypotension, leukopenia, thrombocytopenia, and evidence of hepatic toxicity. Minor changes in serum electrolytes were noted. Coagulation studies were normal. The dose-limiting toxic effect for the high-dose arm was myelosuppression. Median maximum tolerated dose among high-dose strategy patients was 18×10^6 units/m^2, but there was marked interpatient variation. We conclude that both dose schedules were relatively well-tolerated. Because of individual variation in tolerance, high-dose treatment should include a dose escalation strategy. [Cancer Treat Rep 69:743–750, 1985]

Interferons have shown-some antitumor activity in a variety of human neoplasms including: malignant lymphoma (1), leukemia (2), multiple myeloma (3), breast carcinoma (4), ovarian carcinoma (4), renal cell carcinoma (5), Kaposi's sarcoma (6), and malignant melanoma (6). Clinical antitumor activity has now been established, as recently reviewed (7). However, the mechanism of antitumor action is not known and an optimal dose strategy has not been developed. We are examining these questions in a prospectively randomized dose-response study of lymphoblastoid interferon. While other phases of the study continue, the toxicity analysis has been confined to the first 37 patients as reported here. Interpatient differences revealed by our study design should prove to be useful in planning further trials.

METHODS

Interferon

Human lymphoblastoid interferon (Wellferon, Burroughs Wellcome Company, Kent, United Kingdom) was provided by Pacific Isotopes and Pharmaceuticals of British Columbia. This preparation of alpha interferons was extracted from the Namalwa cell line and is supplied at a purity of approximately 85% (8). Further characteristics of this preparation have been previously reported (8). Serum interferon levels were measured using Sindbis as the challenge virus, essentially as described by Larke (9), with the modification that human foreskin fibroblasts were used as target cells. In each set of assays, a laboratory standard, which had been calibrated in terms of the international standard of human leukocyte interferon (69/19), was included as a control. Concentrations of interferon in the sera were expressed in international units on the basis of the value obtained for the laboratory standard. Sera were tested from an initial 1/10 dilution.

Patients

The protocol selection criteria defined a population with well-established disease, since there had to be a mea-

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[2]Presented at the 13th International Union Against Cancer Congress, Seattle, WA, 1982.

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surable component and interferon was not offered as a first-line agent. Patients had to have a performance status of 2 or better (Eastern Cooperative Oncology Group scale) and an expected survival of > 3 months. Patients were excluded if there was evidence of significant sustained hypertension (blood pressure > 150/100 mm Hg), myocardial damage by history or ECG, or cerebrovascular accident. Minor hepatic impairment was allowed, provided the serum bilirubin was normal and alkaline phosphatase, SGOT, and LDH were not greater than twice the upper limit of normal. Acceptable hematologic reserve was defined as a total polymorphonuclear leukocyte count > 1500/mm^3, hemoglobin > 10 g/dl, and platelet count > 125,000/mm^3.

Treatment

Patients were randomly assigned to one of two treatment strategies after preliminary evaluation and informed consent. The low-dose strategy (LDS) consisted of 2×10^6 units/m^2 of interferon daily by im injection, for an induction period of 28 days. These patients remained in the hospital for the first 5 days for observation. If there was no disease progression at 28 days, maintenance treatment was continued daily on alternate weeks until evidence of progression. This schedule was in keeping with other alpha-interferon studies (10,11), so that later comparison might be possible. More importantly, indirect antitumor activity through stimulation of host immunity might be anticipated (12,13).

By contrast, the high-dose strategy (HDS) was based on laboratory data, suggesting that relatively high-dose continuous interferon exposure provided the optimal conditions for direct tumor cytotoxicity (14). Because of conflicting preliminary reports on tolerable dose levels (15,16), an escalating schedule was selected to allow for individual variation in tolerance. HDS patients received interferon by continuous iv infusion over 10 days. The starting dose was 5×10^6 units/m^2/day, with daily escalation of 5×10^6 units/m^2/day to a total of 20×10^6 units/m^2/day, then further escalation every other day. Patients showing no disease progression at 28 days continued receiving interferon infusions for the first 10 days of each 28-day cycle until progression. On Day 1 of the second and each subsequent treatment cycle, patients received one-half the maximal tolerated dose of the previous treatment followed by escalation to the previous maximal tolerated dose on the second day if $< 20 \times 10^6$ units/m^2 or on the third day if $> 20 \times 10^6$ units/m^2. Further escalation continued as described above.

Dose reduction on both treatment arms was keyed to peripheral blood counts. Interferon dose was reduced by 50% for polymorphonuclear leukocyte counts < 800/mm^3 or for platelet counts < 100,000/mm^3, and treatment was interrupted for polymorphonuclear leukocyte counts < 500/mm^3 or platelet counts < 50,000/mm^3. Reduction or interruption of treatment could also be at the discretion of the treating physician for unexpected toxicity.

Patient Monitoring

Patients were closely monitored during the first day of treatment with measurement of vital signs (temperature, pulse, and blood pressure) just before treatment; then hourly for 6 hours; every 3 hours for 6 hours; and every 4 hours for 12 hours. A hematologic panel of tests (total wbc count with differential, platelet count, hemoglobin, prothrombin time, and partial thromboplastin time) and a serum biochemical panel (total bilirubin, SGOT, LDH, alkaline phosphatase, creatinine, electrolytes, and calcium) were evaluated at 12 hours. During the remainder of the first treatment vital signs were repeated every 4 hours while the patients were awake and in the hospital. LDS patients had hematology and biochemistry panels repeated three times per week during treatment, while HDS patients had these studies repeated daily while receiving treatment and on Days 15 and 22 between treatments. Serum for interferon levels was routinely obtained prior to each treatment cycle and on Day 7 or 8 of treatment. For LDS patients the latter sample was obtained between 4 and 6 hours after daily im injection. HDS patients were sampled during continuous iv infusion. A single HDS patient had additional samples analyzed during the first 10 days of treatment.

RESULTS

Thirty-seven patients had been entered in the study > 3 weeks before this analysis and were therefore considered evaluable for toxicity. This toxicity analysis includes four patients who failed to complete the first 10 treatment days and were classified as inevaluable for assessment of antitumor effect. In all, 43 HDS and 48 LDS courses of treatments were given. There were four males and 33 females, with a median age of 54 years (range, 20–71) and a mean performance status of 0.9. Thirty-one patients had been previously treated with radiotherapy, while only two had not received chemotherapy. In the latter patients, interferon was offered only after more conventional anticancer drugs were refused. The histologic diagnoses included 25 patients with breast carcinoma, eight with ovarian carcinoma, two with non-Hodgkin's lymphoma, one with osteogenic sarcoma, and one with nasopharyngeal carcinoma. A summary of patient characteristics by dose is shown in table 1.

Interferon Levels

For LDS patients receiving the full dose of 2×10^6 units/m^2, serum interferon levels varied from < 10 to 300 units/ml, with a median of 60. HDS patients were analyzed individually to allow for dose-related interpatient

TABLE 1.—Patient characteristics*

Characteristic	LDS	HDS
Median age in yrs	51	57.5
Sex		
Male	3	1
Female	18	15
Mean performance status	1.1	0.7
Previous treatment		
Chemotherapy	20	15
Radiation therapy	18	13
Histologic cell type		
Breast carcinoma	13	12
Ovarian carcinoma	5	3
Non-Hodgkin's lymphoma	1	
Nasopharyngeal carcinoma	1	
Osteogenic sarcoma	1	

* Unless otherwise specified, values = No. of patients.

differences. The relationship between dose and response for the patient with the greatest number of determinations is shown in figure 1. These results demonstrate the presence of biologically active agent in patients' serum and are in keeping with reports by others (2,17,18).

Toxic Effects

Fever.—Every patient developed some increase in body temperature while receiving interferon. Since the kinetics of untreated fever have been reported by others (15), acetaminophen was routinely used for symptomatic relief in our patients. Although symptoms were generally well-controlled, a dose response was evident. During 35 courses in 14 LDS patients, oral temperatures did not exceed 38°C, while this was only true for three courses in two HDS patients.

Fatigue.—Lassitude was a common problem. Since there was wide individual variation, a simple scoring system was designed for quantitation: 0 = no fatigue; 1 = mild fatigue, usually elicited only on questioning and not resulting in increased bedrest; 2 = moderate fatigue sufficient to interfere with more ambitious daily activities and leading to increased bedrest; and 3 = severe fatigue interfering with all daily activity and associated with bedrest for > 50% of usual waking hours. The wide individual variation in fatigue is shown by the comparison of HDS and LDS patients in figure 2. Although more HDS patients experienced severe fatigue, this was not statistically significant and there was no overall dose response. Fatigue was sufficiently marked in two patients to require treatment interruption.

Gastrointestinal dysfunction.—All patients admitted to at least mild anorexia. In some patients this was difficult to differentiate from mild fatigue. When vomiting did occur it tended to be sporadic during interferon administration and relatively mild in intensity compared to the more active emesis frequently seen with many chemotherapy agents. Treatment was discontinued because of persistent vomiting in only one patient; this patient had LDS treatment. Figure 3 shows the number of patients who experienced nausea and/or vomiting at some time during treatment. The incidence was not related to dose. However, diarrhea was dose-related. Although seven of 21 (33%) HDS patients had diarrhea at some

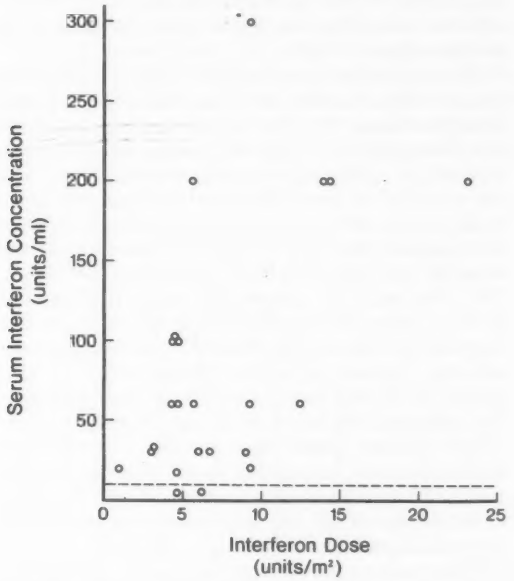

FIGURE 1.—Relationship of administered iv dose to serum interferon level in a single representative patient (P = 0.01, linear regression analysis). Assay sensitivity was 10 units/ml, as indicated by the area below the interrupted horizontal line.

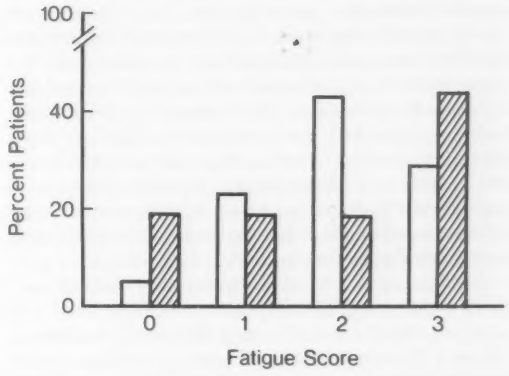

FIGURE 2.—% of LDS (clear bar) and HDS (shaded bar) patients with each numerical score. As detailed in text, fatigue is scored as: 0= none, 1 = mild, 2 = moderate, and 3 = severe. There were 21 LDS and 16 HDS patients.

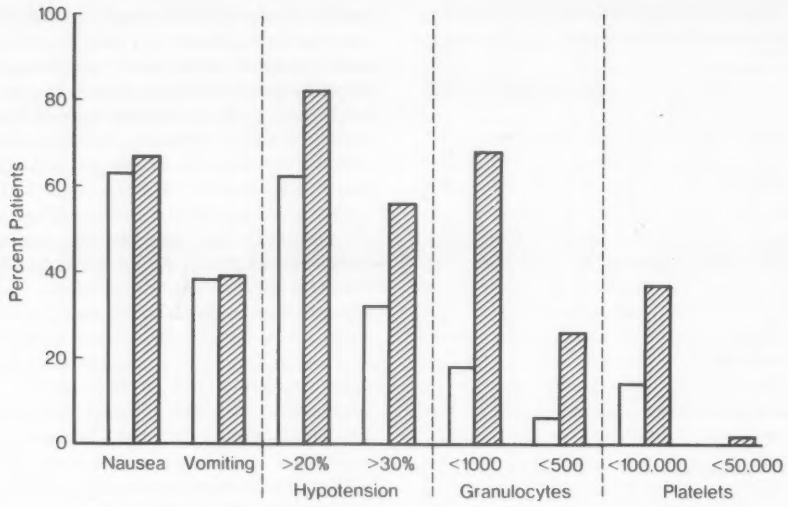

FIGURE 3.—% of LDS (clear bar) and HDS (shaded bar) patients exhibiting the noted toxicity at some time during clinical observation. There were 21 LDS and 16 HDS patients. Hypotension is expressed as % reduction of either systolic or diastolic pressure from pretreatment values. Granulocyte and platelet counts are expressed as cells/mm^3.

time during treatment, this was experienced by only one of 16 (6%) LDS patients ($P = 0.05$; chi-square test). In each patient diarrhea was relatively mild, there was no problem with fluids or electrolytes, and no remedial medications were required. Biochemical evidence of interferon-induced hepatic impairment was common. Twenty of the 37 patients had pretreatment evidence of hepatic impairment, with SGOT values above the upper limit of normal. The remaining 17 patients (nine with HDS and eight with LDS) had no pretreatment evidence of hepatic pathology. Of these patients, 16 (94%) had some SGOT values above normal limits during treatment. Seven of the 16 patients (44%), including five with HDS and two with LDS, developed elevations more than three times the upper limit of normal on at least one occasion. In no patient was the hepatopathy symptomatic, and no patient had a rise in serum bilirubin > 1.5 g/dl. The relationship of SGOT to interferon administration is illustrated for a representative patient in figure 4. Although SGOT values increased significantly with each 10-day course of interferon, there was a return to normal or near normal by the time of the next treatment.

Cardiovascular effects.—Hypotension, defined as a ⩾ 20% decrease in either systolic or diastolic blood pressure, was fairly common among all patients as shown in figure 3. There was a trend for more marked hypotension (> 30% decrease) among HDS than LDS patients. Blood pressure ⩽ 70/40 mm Hg were recorded in two patients, both on the HDS treatment arm. This marked decrease in blood pressure was transient for one patient; in the other patient treatment was interrupted and blood pressure returned to normal within 4 hours. In each patient, clinical examination revealed good peripheral perfusion, with no apparent central nervous system effects or decrease in urinary output.

Hematologic toxicity.—Figure 3 shows the incidence of granulocytopenia. HDS treatment clearly induced more granulocytopenia ($P < 0.01$; chi-square test). Treatment was interrupted in 17 patients, as dictated by protocol. Granulocyte counts subsequently returned toward normal within 24-48 hours. No patient had persistent leukopenia and no septic episodes could be attributed to interferon-induced granulocytopenia. Thrombocytopenia was recorded less frequently than granulocytopenia (fig 3). The effect was more pronounced among HDS patients ($P = 0.1$; chi-square test). Treatment was interrupted in one patient, as dictated by protocol. No patient developed petechiae, purpura, or related bleeding while receiving interferon. Falling hemoglobin was not a clinical problem, although mild decreases during treatment were recorded. Between pretreatment and Day 22 of the first treatment course, hemoglobin varied from baseline values by -3.2 to + 3 g/dl, with a median of -0.7. The trend of decreasing values was only statistically significant among LDS patients ($P = 0.01$; Wilcoxon test).

Central nervous system.—At the time of data review there had been no clear episodes of central nervous system impairment. However, since closing this phase of study, one patient developed cerebellar ataxia and mild confusion while receiving a relatively high interferon

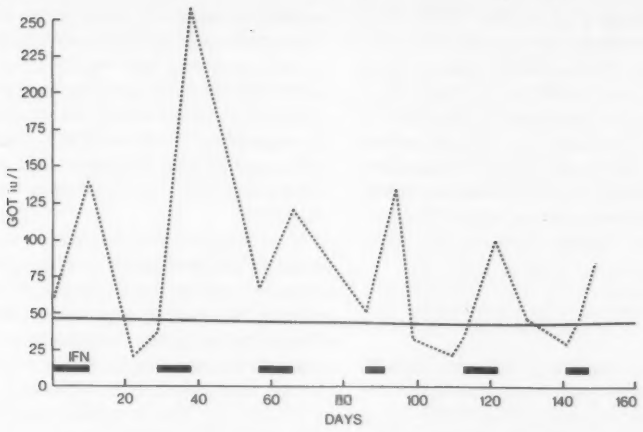

FIGURE 4.—Serial SGOT values in 1 patient. Upper limit of normal is 47 IU/L as indicated by the continuous bar. Interrupted heavy bars denote the periods of interferon infusion.

dose similar to that reported by others (19). Concurrent electroencephalogram (EEG) revealed a moderate excess of 15–20 cps fast activity in all regions, some low-voltage semirhythmic irregular 2–4 cps slow activity seen most prominently in temporal and posterior areas, and poorly sustained alpha rhythm. All symptoms and EEG findings returned to normal within 1 week of treatment interruption.

Minor side effects.—There were relatively small fluctuations of serum electrolytes while receiving interferon. This is demonstrated by a comparison of the induction pretreatment and Day-8 values for both HDS and LDS patients. Although the fluctuations during this period for serum sodium varied from −10 to +5 mEq/L (median, 2), there was a significant overall decrease in values for both LDS ($P < 0.001$; Wilcoxon test) and HDS ($P = 0.025$) patients. There was also a trend for decreasing serum potassium: $P = 0.22$ for HDS patients and $P = 0.11$ for LDS patients. Changes in serum calcium ranged from −1.4 to +0.3 mg/dl (median, −0.7). The decline in calcium was significant for both LDS ($P < 0.025$) and HDS ($P < 0.001$) patients and tended to be more marked among HDS patients ($P = 0.08$; Wilcoxon test). Although such minor shifts in fluids and electrolytes may be indirect events, it is of interest that much higher doses of interferon can produce clinically significant hypocalcemia (2). Diminution of taste sensation was common as was the sensation that a metallic taste had been imparted to food. A possible related common complaint was xerostomia. Two patients developed mild oral ulceration while receiving treatment. Alopecia developed in the same patient who developed the cerebellar ataxia described above.

Toxic effects not encountered.—No patient developed evidence of renal impairment on routine testing (abnormal creatinine or significant proteinuria). A single episode of pulmonary embolization was not felt to be directly related to interferon administration. This patient had a history of recurrent emboli partially controlled by inferior vena cava plication. The problem may have been aggravated by fatigue-related inactivity and increased bedrest. No other patient demonstrated any abnormality of coagulation while in the study.

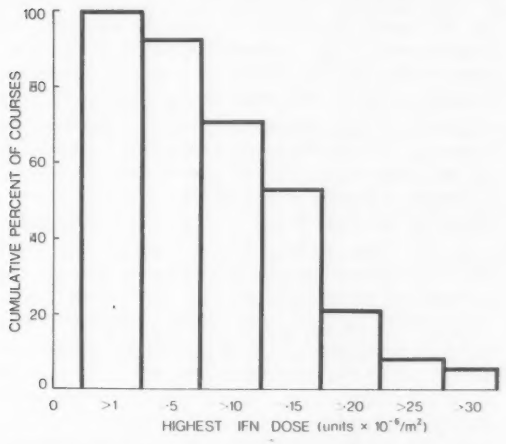

FIGURE 5.—Maximum dose attained in each of 43 high-dose courses. Results are expressed as the cumulative % for each dose range.

Maximum Tolerated Dose

There were 43 high-dose courses given to 16 patients. The maximum dose attained in each course is shown in figure 4, expressed as a cumulative percent. As shown in column 5, in only 21% of the courses was the peak dose

$> 20 \times 10^6$ units/m^2 (median, 18×10^6). There were marked interpatient differences in tolerence to interferon, as shown in figure 5. Although there was a general impression that tolerence was related to age, tumor burden, and history of previous chemotherapy, this was not clearly demonstrated in this study. For example, of the two patients who had not previously received chemotherapy, one was unable to complete her low-dose induction treatment because of myelosuppression, while the other, at age 78, achieved the highest dose in the study (56×10^6 units/m^2).

DISCUSSION

Prospective randomization allowed a clearer view of some of the practical problems of interferon administration than would otherwise be possible. This is illustrated by the two predominant toxic effects. The main dose-limiting toxic effect was defined by reduction of circulating granulocytes. Not only did HDS patients show more granulocytopenia (fig 3), but this was the leading cause of dose interruptions. This was in contrast to the results for fatigue, the main symptomatic complaint. There was no overall difference between treatment arms. However, there were clear interpatient differences. While some LDS patients were incapacitated, other HDS patients had little or no problem (fig 2). This combination of unpredictable susceptibility with an individual dose response suggests caution in initiating treatment at a relatively high dose. Some patients may prove to be inordinately sensitive.

Our experience with gastrointestinal and central nervous system toxic effects was much the same as that of others. Nausea, vomiting, and hepatic dysfunction were more frequent than in some other studies (15,20-22), perhaps reflecting differences in patient populations. As with fatigue, there was wide individual variation (fig 3). Although we noted only mild, short-duration asymptomatic biochemical hepatopathy, severe idiosyncratic reactions have been reported (23). Marked central nervous system dysfunction has been seen by ourselves and others (19). There is now evidence that EEG abnormalities may also appear at relatively low doses, perhaps as a direct effect of interferon on the central nervous system (19).

Hypotension has not been a prominent feature of other reports (15,20,22). In our experience this was frequent and could be quite marked, especially in HDS patients (fig 3). Although there were no complications, this might not apply to patients with more prolonged episodes or underlying vascular disease. The physiologic basis for hypotension is not clear; however, a direct effect on the myocardium is possible (24).

Two toxic effects reported by others have not been seen by us. Renal failure has been documented in experimental animals and more recently has been recognized in occasional interferon patients as well (23,25,26). Since the severe nephropathy appears to be a sporadic event, it is not surprising that we have not encountered it. We have not seen any systematic alteration in frequently monitored routine coagulation studies as has been recently reported by Mirro et al (27) in a study of seven patients with myeloblastic leukemia. This apparent discrepancy may relate more to the disease under study than the treatment.

It is important that our data on maximal tolerated dose refer to the median of maximal doses achieved by patients who showed wide individual variation (5×10^6 to $> 50 \times 10^6$ units/m^2) and were treated with an escalating dose strategy. Using this strategy investigators can expect, on the average, to achieve interferon doses significantly higher than those reported by Priestman (15), but certainly less than has been reported by others (2,17). The value of an escalating strategy is supported by our finding of marked interpatient differences not predictably explained by age, extent of disease, or history of previous chemotherapy. Thus, for any high-dose program, use of a fixed dose will result in one of two problems. Many patients will fail to reach their maximal tolerated dose, or conversely, they will exceed their tolerable dose with significant toxicity and a forced-dose reduction. At least one clinical study was interrupted and then restarted because of this problem (28).

Additional objectives of this interferon study are to identify a possible clinical dose response and to investigate the relationship of in vitro immune function tests to dose, duration of treatment, and treatment response. As already reported by us (29), we were more interested in the longer term effects of interferon than the transient early activity investigated by others (30-32). Briefly, over the 1-week periods of low-dose interferon administration, there was a trend of increasing natural killer cell activity, suggesting immunostimulation. This is in keeping with some other studies (32). Results were quite different over the longer term. During the total time in the study, LDS patients showed no evidence of immunostimulation; in fact, there was an overall statistically significant increase in suppressor-cytotoxic cells and a trend of decreasing helper/suppressor ratios. By contrast, during the same period, HDS patients showed statistically significant increases in natural killer cell activity and a significant decrease in the number of suppressor-cytotoxic cells. Thus, relatively high dose intermittent treatments may take advantage of both direct cytotoxic (14) and indirect immunostimulatory effects of interferon.

Our preliminary tumor response data have been reported (4). As demonstrated by the interim results shown in table 2, tumor responses have been seen in both breast and ovarian carcinomas. Although higher response rates might be expected had interferon been used as a first-line agent, it appears unlikely from these data that interferon will prove to be as active as presently available chemo-

TABLE 2.—Tumor response

Site	Total No. of patients	No. of responses*				
		CR	PR	MR	S	P
Breast	27	0	1	4	5	17
Ovary	15	0	1	3	3	8
Other	4	0	0	0	0	4

*CR = complete response; PR = partial response; MR = minimal measurable response; S = stabilization; and P = progression.

therapy. This is in keeping with other results (33,34). Interferon may prove to be more effective at an earlier stage of disease, with other types of neoplasms, in combination with other treatments, or once an optimal dose strategy has been determined. To date, there is a trend in favor of one of the treatment arms in our study, but this is not yet statistically significant.

The design of further studies will be based on our present experience. In particular, we feel that investigators wishing to achieve relatively high doses should use an escalating dose strategy to allow for wide individual variation in tolerence.

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Action of Oral Methylprednisolone in Terminal Cancer Patients: A Prospective Randomized Double-Blind Study[1]

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A 14-day, randomized, double-blind crossover trial was carried out comparing an oral glucocorticoid, methylprednisolone (MP), against placebo for the relief of pain and other symptoms in 40 terminally ill cancer patients. After the 14-day, double-blind phase was completed, all patients were given MP for 20 days. The daily dose of MP was 32 mg, and end points of the study were pain, psychiatric status, appetite, nutritional status, daily activity, and performance. Mean intensity of pain (visual analogue, 0–100 ± SD) was 36.8 ± 14 after MP treatment and 57.7 ± 15 after placebo ($P < 0.01$). Following the 14-day, double-blind phase, appetite and daily activity increased in 24 of 31 patients (77%) and in 21 of 31 patients (68%) with MP, respectively; depression and analgesic consumption decreased in 22 of 31 patients (71%) and in 16 of 28 patients (57%) with MP, respectively. MP was preferred over placebo by 23 of the 31 patients (74%), and, in 22 of the 31 cases (71%), the investigator chose MP over placebo. No serious toxicity was found at the dose of MP used. It is concluded that MP increases the comfort of terminally ill cancer patients. [Cancer Treat Rep 69:751–754, 1985]

Several studies have suggested that some symptoms of cancer patients, such as anorexia and inactivity, can be alleviated by corticosteroid treatment, giving the patients a sense of relative well-being (1–5). However, only a few parameters were studied, and prospective placebo-controlled trials were not performed on these patients. Recently, the American Cancer Society recommended that studies be pursued on the evaluation of the action, efficacy, and side effects of corticosteroids in terminal cancer patients (6). Therefore, this prospective, randomized, double-blind crossover study evaluated the effects of methylprednisolone (MP) on pain, psychiatric status, appetite, nutritional status, daily activity, and performance in terminally ill cancer patients. In the second phase of the study, all patients received MP for 20 days.

METHODS

Forty terminally ill patients [either defined as such, or those in whom multiple therapeutic techniques (including surgery, radiotherapy, and chemotherapy) had previously failed] were included in this study. Patient characteristics are summarized in table 1. No patients received chemotherapy, radiation therapy, or hormonal treatment(s) during the month preceding this trial. None of the patients were receiving medication, with the exception of oral analgesics (capsules containing a combination of 98 mg of propoxyphene and 400 mg of dipyrone) according to individual requirements. No patients had a history of the following major contraindication(s) for corticosteroid therapy: infection, history of peptic ulcer, insulin-dependent diabetes, or severe psychiatric disturbances. None of the patients had neurological deficits or altered levels of consciousness, and all of them could receive oral medication.

After informed consent was obtained, the patients were included in the 14-day, double-blind phase of the trial. On Day 0, they were randomized to receive oral MP, 32 mg daily (16 mg twice a day), or placebo for 5 days. Days 5–7 were treatment-free. On Day 8, a crossover was made and the treatment was reestablished for Days 8–12. On Day 13, the double-blind phase of the study was completed and all patients were given 32 mg of MP daily for 20 days.

Patients were evaluated in the mornings on Days 0, 5,

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TABLE 1.—Patient characteristics*

Characteristic	Admitted to the trial	Evaluable
Mean age in yrs (mean ± SD)	54 ± 12	51 ± 12
Sex		
Male	18	12
Female	22	19
Primary localization of tumor		
Colon	9	8
Breast	8	7
Lung	6	4
Soft tissue sarcoma	4	4
Kidney	3	2
Prostate	3	2
Head and neck	2	1
Melanoma	2	1
Pancreas	2	1
Ovary	1	1
Total	40	31

* Unless otherwise specified, values = No. of patients.

13, and 33. While 19 patients were in the hospital until completion of the double-blind phase of the trial, the other 21 patients were evaluated as outpatients. For each patient, all of the evaluations were performed by the same investigator.

End points of this study were:

Pain.—All painful localizations were schematically recorded. Intensity of pain was determined by the method of visible analogue (7,8), and by the amount of daily oral analgesic consumption. Only 28 patients were evaluated for pain relief; three reported no pain at the baseline evaluation.

Psychiatric status.—Hamilton's psychometric tests (9) for anxiety and depression were performed.

Appetite.—To assess appetite, two parameters were evaluated: (*a*) the patient's self-evaluation, measured by a visible analogue similar to the one used for the intensity of pain; and (*b*) the mean percentage of food that the patient was reported to eat for each meal. Meals were standardized for all patients. Outpatients were served standardized rations of a diet previously discussed with the relatives. Meals were served in standard plates, glasses, and cups. Patients received no nutritional counseling and they ate ad libitum. The same relative of the patient evaluated the percentage of consumption after each meal. All information was recorded in structured forms.

Nutritional status.—Four parameters were used to assess nutritional status: actual/usual body weight, triceps skinfold thickness, arm muscular circumference, and albuminemia. All parameters were expressed as a percentage of the standard value for each test (10), and the mean value of the four percentages was obtained. When this mean value was > 90 percent, the patient was considered normal; patients with mean values < 90% were considered malnourished.

Activity.—A structured interview was performed with the patient, the nurse, and relatives, regarding the patient's ability to feed, clothe, and care for himself, and his interest in recreational activities and communication. Results were recorded in a score of 0–10. Performance status [Eastern Cooperative Oncology Group (ECOG), Zubrod] was also determined.

Student's paired t test according to the method of Hills and Armitage (11) for crossover trials was used in the case of continuous variables. In the case of analgesic consumption, activity score, and performance status, in which ordinal data might have serious non-normality distribution, the Wilcoxon test was applied according to the method of Koch (12). Yates' corrected chi-square test was used for the analysis of proportion. When the sample size was very small, or the results of the chi-square test fell within the limits of significance, Fisher's exact test was applied.

RESULTS

Nine patients did not complete the trial: three died before the end of the double-blind phase; four had clinical complications that required medication upon admission to the hospital; and two dropped out because of protocol violation. Four of nine patients were dropped from the trial while receiving MP: a patient with renal carcinoma died with neurological symptoms, and no autopsy was performed; a patient with colon carcinoma was admitted with bowel obstruction; a patient with pancreatic carcinoma had sepsis; and a patient with breast cancer had a pulmonary embolism requiring anticoagulation.

Results of the remaining 31 patients after completion of the trial are summarized in tables 2 and 3. No significant period or residual effects were found for any of the features according to the methods of Hills and Armitage (11) and Koch [(12), table 3]. Only 28 patients were evaluated for pain, since three reported no pain at the baseline evaluation. Pain had bone localization in 16 cases, had visceral origin in seven, and was due to nerve compression in five. No differences in response to MP were recorded for the different localizations of pain. As seen in table 2, the intensity of pain (measured by the method of visual analogue and the daily consumption of analgesics) was significantly lower after MP as compared with baseline or placebo. Other parameters that showed significant improvement were depression, appetite, and food consumption. On the other hand, anxiety and performance status (ECOG) did not show significant improvements after 5 or 33 days of MP, as compared to baseline or placebo. Thirty-two of the 40 patients (80%) were considered malnourished after initial nutritional evaluation. No change in nutritional status was observed after either phase of the study.

Table 3 summarizes the responses of patients after

TABLE 2.—Effects of MP on the clinical parameters*

	Intensity of pain (visual analogue, 0–100)	Analgesic consumption (No. of capsules)	Depression score	Anxiety score	Appetite (visual analogue, 0–100)	Food consumption (% of each meal)	Performance status (ECOG)	Activity score
Baseline	57.7 ± 15	3.6 ± 1.5	30 ± 14	14.6 ± 6	26.5 ± 10	43 ± 10	3.5 ± 0.7	3.2 ± 2
Placebo	50.1 ± 15	3.3 ± 1.5	27 ± 10	14.7 ± 7	29.5 ± 15	50 ± 12	3.2 ± 0.6	3.4 ± 2
MP (Days 5–13)	36.8 ± 14	1.8 ± 1.7	19 ± 10	18.8 ± 6.4	40.1 ± 15	65 ± 15	3 ± 0.8	6.7 ± 2.4
MP (Day 33)	4.1 ± 20	2.4 ± 1.3	24 ± 10	17.8 ± 8	33 ± 13	55 ± 13	3.3 ± 0.8	4 ± 2
P values								
Baseline vs placebo	0.06†	NS‡	NS†	NS†	NS†	NS†	NS‡	NS‡
Placebo vs MP (Days 5–13)	< 0.01	< 0.05	< 0.05	NS	< 0.05	< 0.01	NS	< 0.01
Placebo vs MP (Day 33)	< 0.05	NS	NS	NS	NS	NS	NS	NS

* Unless otherwise specified, values = mean ± SD. NS = not significant.
†Student's paired t test (ref 11).
‡Wilcoxon test (ref 12).

completion of the initial, double-blind phase. Response was defined as a favorable difference of ⩾ 30% as compared to placebo. With the exception of anxiety, all parameters, including patient's or investigator's selection, showed differences in favor of MP over placebo.

No differences were found in the baseline parameters between the group that chose MP (23 patients) and the group that chose placebo or did not find any differences between treatments (eight). However, after receiving MP, the results were significantly better in the group that finally chose MP.

Seven of nine patients with breast and prostate cancers (potentially endocrine-responsive tumor) chose MP at the end of the trial (78%), versus 16 of 22 with other tumors (73%, *P* value = not significant).

Five patients died between Days 13 and 33 of the trial. No autopsies were performed. None of the eight patients who had chosen placebo or had found no difference between MP and placebo after the double-blind phase showed significant improvement of symptoms at Day 33.

TABLE 3.—Responses of patients after completion of the double-blind crossover phase of the study*

Feature (No. of patients)	No. of patients (%)		
	Better with MP	Better with placebo	No difference
Pain intensity (28)†	19 (68)	4 (14)	5 (18)
Analgesic consumption (28)†	16 (57)	4 (14)	9 (32)
Appetite (31)	24 (77)	3 (10)	4 (13)
Activity (31)	19 (61)	5 (16)	7 (23)
Depression (31)	22 (71)	4 (13)	5 (16)
Anxiety (31)	6 (19)	7 (23)	18 (58)
Patient's selection (31)	23 (74)	3 (10)	5 (16)
Investigator's selection (31)	22 (71)	4 (18)	5 (16)

* Response was defined as a favorable difference of ⩾ 30% as compared to placebo.
†Only 28 patients were evaluated for pain; 3 reported no pain at baseline.

One of the 23 patients who had chosen MP after the initial double-blind phase died; 12 of the remaining 22 patients (55%) showed no differences between values observed after the initial double-blind phase and those observed after the second (20-day) phase of the study. In the other ten patients (45%), values at Day 33 were significantly lower or did not differ from the baseline results.

Two patients developed cushingoid facies, and another two showed significant enhancement of their anxiety, but all were able to complete the treatment. One patient showed signs of mild fluid retention during the second phase of the trial. No cases of upper gastrointestinal bleeding were observed. Tumor regression was not detected in any patients.

DISCUSSION

We studied the efficacy of MP in a prospective, randomized trial in terminally ill cancer patients. We used a 14-day, double-blind, crossover protocol to test the active drug against placebo for a short time period to avoid biased results that might appear with longer treatments in the rapidly progressive stage of the disease. After the double-blind crossover phase, we also evaluated parameters such as nutritional status and adverse effects.

The oral administration of 32 mg/day of MP resulted in definite patterns of response for each of the different parameters. Pain (evaluated by visual analogue and analgesic consumption) was the end point most sensitive to MP treatment, followed by depression, appetite, and food consumption. Appetite, as well as well-being, has recently been reported to enhance with prednisolone in a double-blind crossover trial (13). Two end points, anxiety and performance status, were insensitive to MP treatment. All parameters sensitive to MP reached maximum improvement during the first phase of study.

The number of patients who responded better to the ac-

tive drug also confirmed the beneficial effects of MP in all parameters, with the exception of anxiety (table 3). Of eight patients who had not experienced significant relief of symptoms after the initial double-blind phase of the trial, none showed any significant improvement at Day 33. This suggests that MP exerts its action rapidly, and that the chances of obtaining better responses after 5 days of treatment are poor.

Although no significant differences were recorded between the beneficial effects of MP at Day 5 and at Day 33, most parameters had deteriorated slightly by Day 33 as compared to Day 5, in spite of a significant improvement at Day 33 as compared to baseline. Eight of the 23 patients (34%) who initially responded significantly to MP showed disappearance of the symptomatic benefits of the drug by Day 33. The present study cannot establish whether this is due to a loss of the effect of MP with time or to the natural evolution of the disease.

In 1974, Moertel et al (14) published results comparing dexamethasone at 0.75 and 1.5 mg four times daily to placebo in 116 patients with advanced gastrointestinal cancer. They also found a significant symptomatic improvement (appetite and strength) for patients receiving dexamethasone after 2 weeks of treatment, but such improvement disappeared after 4 weeks of treatment. In their trial, toxicity was also low, with only one case of gastrointestinal hemorrhage.

The mechanism of action of MP in patients with terminal cancer is not clear, but it might be ascribed to its euphoriant activity (3,15,16) or to the inhibition of prostaglandin metabolism (17). Although the number of patients was small, MP treatment was not associated with marked toxicity in any patients. However, prolonged corticoid treatment has been associated with weakness, delirium, osteoporosis, and immunosuppression, and all of these features are commonly present in advanced cancer patients (6–16).

Although more trials are needed to establish the optimal dose and modality of administration of MP, we consider that short courses of corticoids (eg, 2 weeks) can be given to severely symptomatic advanced cancer patients who have no major contraindications.

Finally, the importance of conducting placebo-controlled trials is emphasized by the significant rate of response obtained with placebo for many parameters in this study. Further studies are required to determine optimal dose, schedules, and mode of administration of MP in terminal cancer patients.

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Mitomycin and Tamoxifen Versus Mitolactol, Doxorubicin, and Tamoxifen in Patients With Previously Treated Breast Cancer[1,2]

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One hundred thirty patients with previously treated advanced breast cancer were treated in a cooperative trial to investigate the effect of mitomycin plus tamoxifen (MT) and to compare its relative value to combination therapy with mitolactol, doxorubicin (ADM), and tamoxifen. An additional 76 patients who had had prior exposure to ADM were randomized to receive MT or MT plus fluoxymesterone. MT versus mitolactol, ADM, and tamoxifen was not significantly different in efficacy; however, there were significant differences in toxicity. This study showed that MT is an effective and acceptable second-line treatment for advanced breast cancer. [Cancer Treat Rep 69:755–760, 1985]

The combination of mitolactol (DBD), doxorubicin (ADM), and tamoxifen (TAM) (DAT) has been shown to be an effective treatment in patients with previously treated breast cancer (1,2). In a pilot study comparing DAT to mitomycin plus TAM (MT), the latter was found to be an effective second-line treatment for metastatic breast cancer (3). For this reason, the present cooperative group trial was initiated. The Eastern Cooperative Oncology Group (ECOG) undertook a study, investigating second-line treatment for advanced breast cancer, comparing DAT to MT (in patients who had not received prior ADM). Further, the relative value of MT versus MT plus fluoxymesterone (MTF) was investigated in patients who had had prior ADM therapy. It was postulated that fluoxymesterone might allow a larger dose of mitomycin to be administered, with a resultant longer time to treatment failure of the responders.

MATERIALS AND METHODS

From May 1979 to May 1981, 130 patients were randomized to receive DAT versus MT. From May 1979 to December 1981, 76 patients were randomized to receive MT versus MTF.

Patient Selection

Patients with histologically documented diagnosis of recurrent or metastatic breast carcinoma were eligible for the protocol, provided that the patients had an ECOG performance status of 0, 1, 2, or 3 (4), had had prior exposure to at least one chemotherapy regimen (possibly as adjuvant therapy), and had had no previous exposure to mitomycin or TAM. Patients randomized to DAT versus MT had had no prior ADM or DBD. Patients randomized

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to MT versus MTF had had no prior fluoxymesterone but should have had prior ADM. Patients with prior medical hormonomanipulation were eligible (*a*) if progressive disease was demonstrated within 12 weeks of initiating hormonotherapy and no withdrawal improvement was seen 7 days after discontinuing hormones, or if there was some withdrawal improvement, there had been subsequent progression, or (*b*) if hormonotherapy was discontinued after relapse and further disease progression was documented over at least a 10-day period of observation off therapy. Patients with prior surgical hormonomanipulation were eligible if 3 weeks had elapsed since surgery and disease progression was documented. In the case of osseous-only metastases, disease progression had to be associated with increasing analgesic requirements and/or decreased performance status and/or an elapse of at least 12 weeks since surgery. Patients had measurable and/or evaluable disease, wbc count $\geq 4000/mm^3$ and platelet count $\geq 100,000/mm^3$ (unless there was documented bone marrow involvement), and adequate renal and hepatic functions unless the abnormality was due to metastatic involvement. The patients did not have a nonmalignant systemic disease or psychiatric risks that precluded their receiving any of the treatment options or a history of other malignant neoplasms. The patients signed an institutional informed consent form.

Treatment Regimens

For North American patients, treatment was assigned by a central office using randomized blocks of size 4 within each of 20 cells formed by the strata: (*a*) ECOG performance status of 0 or 1 versus 2 or 3; (*b*) dominant metastasis to the liver (dire visceral), nodular lung with more than six nodules, lymphangitic lung, central nervous system, and/or bone marrow with wbc count $< 4000/mm^3$ and/or platelet count $< 125,000/mm^3$ versus all other metastases; and (*c*) hormone responsiveness [prior hormone response vs prior disease progression with hormonotherapy vs unknown prior hormone response and estrogen receptor (ER)-positive vs unknown prior hormone response and ER-negative vs unknown prior hormone response and unknown ER].

Institutional balancing was maintained so that treatments were never unbalanced by more than two patients within any of the 26 institutions that participated. South African patients were not stratified but were randomized by sealed envelopes.

Patients with no prior exposure to ADM or DBD will be referred to as Stratum A. They were randomized to receive either DBD (135 mg/m^2 orally on Days 2-11), ADM (40 mg/m^2 iv on Day 1), and TAM (20 mg orally daily continuously in 28-day cycles) (DAT) or mitomycin (12 mg/m^2 iv on Day 1) and TAM (20 mg orally daily continuously in 35-day cycles) (MT). After a cumulative ADM dose of 475 mg/m^2 had been given, the DAT patients were placed on mitomycin (5 mg/m^2 iv on Day 1), DBD (135 mg/m^2 orally on Days 2-11), and TAM (20 mg orally daily continuously in 35-day cycles) (MDT).

Patients with prior exposure to ADM or DBD and no prior exposure to fluoxymesterone will be referred to as Stratum B. They were randomized to receive either MT (as above) or MT plus fluoxymesterone (20 mg orally daily continuously).

Dosage Modifications

If the wbc count was $< 4000/mm^3$ or the platelet count was $< 100,000/mm^3$ at the end of any cycle, the next cycle was delayed until the counts exceeded these figures or until 2 weeks had elapsed, whichever occurred first. If the counts were still depressed after this delay, then ADM, DBD, and mitomycin were given at 50% of the calculated dose if the wbc count was between 3000 and $4000/mm^3$ and/or the platelet count was between 75,000 and $100,000/mm^3$. The DBD reduction was in number of days rather than in dose per day. If the counts were lower than this after the delay, then ADM, DBD, and mitomycin were not given in the next cycle. In addition, even if no delay was necessary or the counts were completely recovered within 2 weeks, these three drugs were given at 75% of the calculated dose in any cycle following a wbc count nadir $< 2000/mm^3$ and/or a platelet count nadir $< 50,000/mm^3$. If such toxicity occurred in two cycles, the doses of the three drugs were permanently reduced to 75% of the calculated dose. If there was persistent blood cell count depression on mitomycin necessitating a delay of 2 weeks on two successive cycles, then the mitomycin dose could be permanently reduced to 8 or 5 mg/m^2, if necessary. In July 1980, an addendum to the protocol alerted investigators to recent literature reports of interstitial pneumonia following mitomycin. Investigators were advised to permanently discontinue mitomycin if the patient became symptomatic.

The criteria for response and toxicity were those of ECOG (4).

Statistical Methods

Analysis of distribution of prognostic factors and toxicity by treatment used Fisher's exact test (5). Response percentages were analyzed with a multivariate logistic model (6). Treatment differences in distributions of times were assessed with the log-rank test (7). Tests for the effect of various prognostic factors, time to treatment failure, and survival came from the asymptotic normality of parameter estimates in a multivariate proportional hazards model (8). Median survival times and survival curves were obtained with the Kaplan-Meier life-table method (9). Total doses were compared using the Wilcoxon two-sample test (10). Confidence intervals on response percents were exact.

RESULTS

Stratum A

Patient Characteristics

One hundred thirty patients with no prior ADM or DBD were entered in Stratum A between May 1979 and May 1981, at which time accrual to this stratum was closed because of observed differences in times to failure. One hundred thirteen patients in this stratum were considered eligible. Six of the DAT patients were in the study long enough to receive the maximum cumulative dose of 475 mg/m^2 of ADM, at which time mitomycin was substituted for ADM.

Covariates of characteristics of the 113 eligible patients were fairly evenly distributed with respect to performance status, age, ER status, menopausal status, disease-free interval (DFI), time since first recurrence, dominant metastatic site, number of metastatic organ sites, and prior therapy. The metastatic sites were evenly balanced.

Response

Table 1 shows the frequency of response in each regimen. Neither complete response nor complete plus partial response rates were significantly different on the two treatment arms ($P = 0.50$ and $P = 0.32$). There were no significant interactions between treatment and any other patient characteristics. Response was significantly associated in a logistic model with the following characteristics: ER-positive (56%) versus ER-negative (23%) versus ER-unknown (39%); DFI < 1 month (32%) versus 1-24 months (20%) versus > 24 months (48%); skin metastases present (42%) versus none (28%); one to three organs with disease (36%) versus more than four (15%); and age < 60 years (28%) versus ≥ 60 years (50%). The overall median time to first response was 1.5 months on DAT and 1.4 months on MT. Twenty-five of the 38 responding patients have relapsed, with an overall median response duration of 6.9 months. The median response duration on DAT was 7.7 and on MT was 4.1 months ($P = 0.16$).

Time to Treatment Failure

Time to failure was defined as the time to relapse or disease progression, whichever occurred first. Median times to failure on DAT and MT were 3.7 and 2.9 months (fig 1). This difference is not significant ($P = 0.12$). Patient characteristics significantly ($P < 0.0001$) associated with shorter time to treatment failure were DFI (1 month to 2 years) and ER-negative disease.

The DAT patients received a median of three cycles; the MT patients received a median of two cycles. However, it must be remembered that the duration of DAT cycles was 28 days, while the duration of MT cycles was 35 days. The median dose per meter squared of ADM was 92% of protocol-specified dose; the median dose per meter squared of DBD was 82% of protocol-specified dose; and the median dose per meter squared of mitomycin was 94% of protocol-specified dose. The total amount of TAM received by DAT patients did not differ significantly from that received by MT patients.

TABLE 1.—Stratum A response*

Response	No. of responses (%)	
	DAT	MT
Complete	6 (10)	3 (6)
Partial	17 (28)	12 (23)
Stable disease	20 (33)	19 (36)
Disease progression	12 (20)	15 (28)
Unevaluable	5 (8)	4 (8)

*95% confidence interval on complete + partial response was 26%-52% in DAT patients and 17%-42% in MT patients.

Survival

There was no significant treatment difference in survival; median survival times were 9.7 and 7.6 months for patients receiving DAT and MT, respectively ($P = 0.25$; fig 2). In a proportional hazards model, variables significantly associated with a shorter survival were DFI < 2 years ($P < 0.0001$), negative ER status ($P < 0.0001$), three or more organ sites ($P = 0.001$), visceral- or osseous-dominant disease ($P = 0.01$), time since first recurrence < 1 month ($P = 0.02$), and age < 60 years

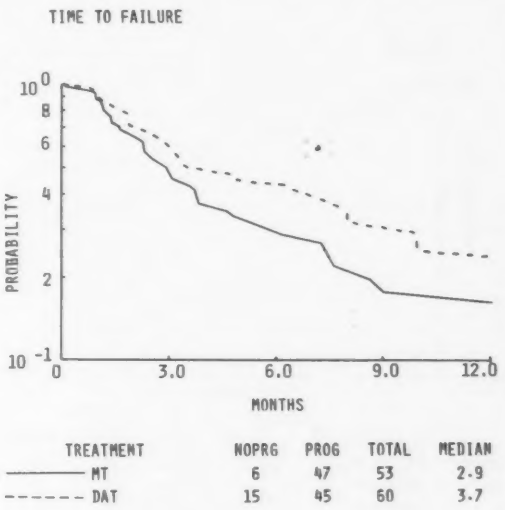

FIGURE 1.—Time to treatment failure in Stratum A.

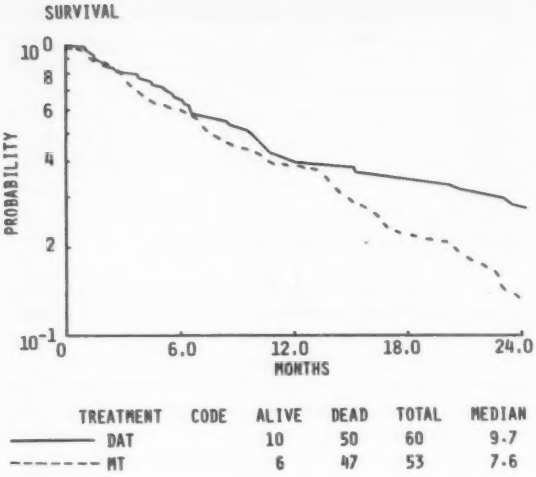

FIGURE 2.—Survival in Stratum A.

($P = 0.03$). Figure 3 shows the effect of ER status on survival.

Toxicity

DAT was associated with significantly more nausea and vomiting ($P = 0.0001$), more alopecia ($P < 0.0001$), more severe leukopenia ($P = 0.02$), and less severe thrombocytopenia ($P = 0.03$). The most important toxic effects were hematopoietic. Anemia occurred in 55% of patients receiving DAT and in 49% receiving MT, leukopenia occurred in 82% receiving DAT and in 70% receiving MT, and thrombocytopenia occurred in 49% receiving DAT and in 60% receiving MT.

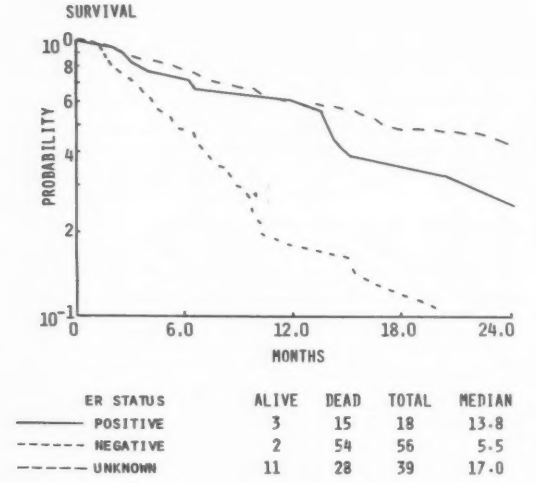

FIGURE 3.—Survival by ER status in Stratum A.

Stratum B

Patient Characteristics

Seventy-six patients were entered in Stratum B between May 1979 and December 1981, at which time accrual to this stratum closed due to slow accrual. Thirty-eight patients were randomized to receive MT and 38 to receive MTF.

Response

Table 2 shows the frequency of response in each regimen. Neither the complete response nor the complete plus partial response rates were significantly different on the two treatment arms ($P = 0.99$ and $P = 0.56$, respectively). Poor performance status was significantly associated with a poorer probability of response. There were no significant interactions between treatment and any patient characteristics.

Time to Treatment Failure

Median times to failure on MT and MTF were 3.3 and 3.4 months. This difference was not significant ($P = 0.94$). Characteristics associated with significantly shorter times to failure in a proportional hazards model were negative ER status; absence of bone metastases; no prior hormonotherapy or no response on prior hormonotherapy; DFI of 1–24 months; performance status of 1, 2, or 3; and time from first recurrence < 2 years. There were no significant interactions between treatment and characteristics.

To investigate the effect of at least 3 months' exposure to fluoxymesterone, the median time to treatment failure for those patients in the study for > 3 months was analyzed. The time to treatment failure was 6.8 months on MT and 4.9 months on MTF ($P = 0.43$; fig 4).

TABLE 2.—Stratum B response*

	No. of responses (%)	
	MT	MTF
Complete	2 (6)	2 (6)
Partial	6 (17)	4 (11)
Stable disease	12 (34)	18 (50)
Disease progression	10 (29)	7 (19)
Unevaluable	5 (14)	5 (14)

* 95% confidence interval on complete + partial response was 10%–40% in MT patients and 6%–33% in MTF patients.

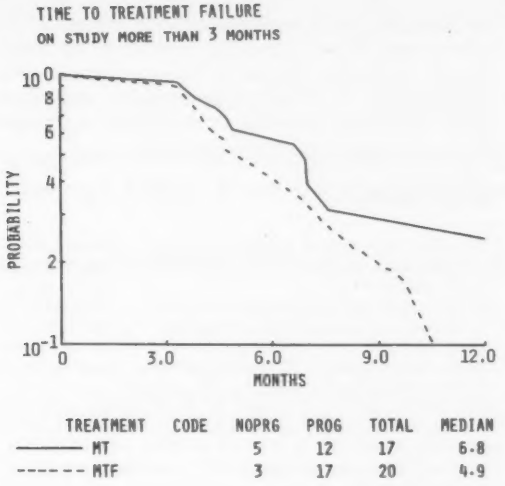
TIME TO TREATMENT FAILURE
ON STUDY MORE THAN 3 MONTHS
PROBABILITY
MONTHS
TREATMENT CODE NOPRG PROG TOTAL MEDIAN
MT 5 12 17 6.8
MTF 3 17 20 4.9

FIGURE 4.—Time to treatment failure for patients in study > 3 mos in Stratum B.

Survival

There was no significant treatment difference in survival; median survival times were 4.6 and 5.9 months for patients receiving MT and MTF, respectively ($P = 0.78$; fig 5). In a proportional hazards model, variables significantly associated with a shorter survival were no response to prior hormonotherapy; two or more sites; performance status of 1, 2, or 3; > 0 DFI; and age < 50 years. There were no significant interactions between treatment and patient characteristics.

Tolerated Dose and Toxicity

The MT patients received a median of two cycles; the MTF patients received a median of three cycles. This difference was not significant ($P = 0.19$). Neither total dose nor dose per meter squared nor dose per meter squared per cycle of mitomycin differed significantly in the two regimens. Total TAM did not differ significantly in the two regimens. The median dose per meter squared of mitomycin was 88% and 93% of protocol-specified dose for MT and MTF, respectively.

Toxic effects were not significantly different for the two regimens. A surprisingly high incidence of nausea and vomiting (26%), although mild to moderate, was reported by investigators. A surprisingly low incidence of virilization (or voice change or hirsutism) was reported for the patients receiving fluoxymesterone (8%). The most important toxic effects were hematopoietic. Anemia occurred in 45% of the patients receiving MT and in 49% receiving MTF, while leukopenia occurred in 59% receiving MT and in 61% receiving MTF. Thrombocytopenia was the most significant hematopoietic toxic effect, with 60% of the patients receiving MT (life-threatening in 9%) and 60% receiving MTF (life-threatening in 8%). No renal side effects were reported in any of the patients receiving MT, and mild to moderate renal toxicity was reported in 3% receiving MTF.

DISCUSSION

Salvage therapy for patients with metastatic breast cancer remains a difficult problem. The choice of second-line drugs is limited because of putative resistance to drugs used initially. There is a need to develop salvage therapy utilizing non-ADM-containing regimens. Pilot studies suggested that the combination of mitomycin and TAM is effective in patients who failed previous chemotherapy, including ADM-containing regimens. DAT has been previously shown to be an effective second-line treatment for metastatic breast cancer if it did not result in significantly better response rates than MT in a pilot study. This large cooperative study confirmed that MT, while causing less subjective toxicity, resulted in similar disease control of patients with advanced breast cancer. Further, as was shown by these results, various patient discriminants were of greater significance in affecting the outcome of treatment. With 113 patients in this part of the study, there was an 80% chance of detecting a significant difference in response rates if the true differences were ≥ 26% (eg, 30%–56%). With 97 patients having died, there is an 80% chance of finding a difference in survival time if this differed between treatments by 67% (ie, median survival of 8 and 13.4 months). These calculations assume a two-sided test of size 0.05 and

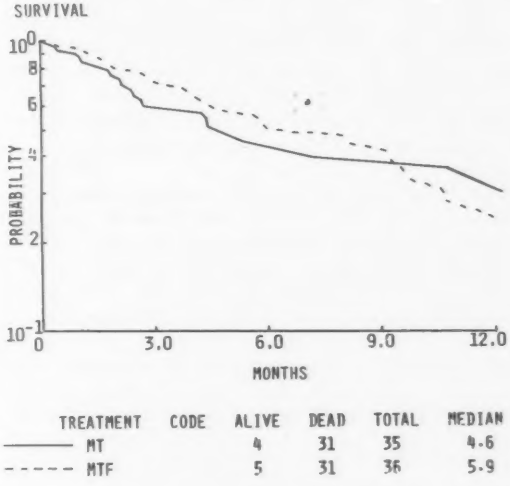

FIGURE 5.—Survival in Stratum B.

approximately exponential distributions. The subjective toxicity of these two treatments differed greatly in that the nausea and vomiting and alopecia were common with DAT treatment and relatively seldom seen (and never severe) with MT treatment. MT therefore has a higher patient acceptability. There was approximately the same amount of hematopoietic toxicity with DAT and MT. It would appear that MT is equivalent to DAT as second-line treatment for breast cancer and could be considered for investigation as a front-line treatment for metastatic breast cancer. Statistical modeling, however, suggested that the response to DAT was superior to that of MT. There are rarer side effects of mitomycin, such as microangiopathic hemolytic anemia and renal failure, but only 2% of the patients receiving MT had mild to moderate renal changes and 9% of the patients receiving DAT had renal changes.

In Stratum B of this study (MT vs MTF), the addition of fluoxymesterone to MT did not result in less hematopoietic toxicity. The addition of fluoxymesterone resulted in a low incidence of virilization.

This study failed to show that MT differed statistically significantly from DAT with respect to response rate, time to treatment failure, or survival as a second-line treatment for advanced breast cancer. It did not show an advantage for adding fluoxymesterone to MT in patients with prior ADM exposure. The data collected and analyzed provide convincing (albeit negative) answers to the questions asked regarding relative efficacy of the various regimens as second-line treatments. The importance of prognostic variables on response adds to better understanding of different discriminants and should serve as a future guideline in developing therapeutic strategies for patients with breast cancer.

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Cooperative Trial of Immunotherapy for Nasopharyngeal Carcinoma With Transfer Factor From Donors With Epstein-Barr Virus Antibody Activity[1,2]

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A prospectively randomized, double-blind clinical trial was conducted to evaluate the effect of immunotherapy with transfer factor (TF) as an adjunct to radiotherapy of patients with stage III nasopharyngeal carcinoma (NPC). The TF was derived from normal young adults with a proven history of infectious mononucleosis and from normal blood donors with elevated antiviral capsid antigen antibody activity. TF prepared in this fashion was previously shown to convert the leukocytes of patients with NPC to a reactive state in vitro and when administered to NPC patients in vivo was associated with apparent slowing of tumor growth, marked lymphocytic infiltration of the tumor, and reconstitution of delayed cutaneous hypersensitivity reactions. From 1974 to 1977, 100 patients with NPC were entered in the study; one-half of the patients were treated with radiotherapy alone and one-half received radiotherapy and an 18-month course of TF immunotherapy. The patients were followed for at least 5 years. No significant difference in disease-free survival or survival was noted between the two groups of patients. The use of this particular preparation and dose schedule of TF in patients with NPC with regional disease was devoid of any antitumor activity. [Cancer Treat Rep 69:761–767, 1985]

Transfer factor (TF) is a nonantigenic, nonantibody, low molecular weight, dialyzable product of sensitized lymphocytes. It was discovered by Lawrence (1) and has been postulated as a mediator of cellular immunity to a variety of antigens (2). TF has been used for the immunotherapy of malignant disease, with some reports of tumor regression (3–10). A major problem with this approach has been the identification of suitable donors of tumor-specific TF (11,12).

Previously, we reported on the treatment of patients with nasopharyngeal carcinoma (NPC) using TF obtained from patients with a past history of infectious mononucleosis (IM) (4,12). The rationale for this form of therapy is that Epstein-Barr virus (EBV) is suspected to play an etiologic role not only in IM (13–16) but also in NPC (17–20). A close and regular serologic association has been noted between the virus and NPC (17,18). Both EBV nuclear antigen (19,20) and DNA (20) have been demonstrated in the nuclei of NPC cells of the poorly differentiated and undifferentiated types; none of the patients in this study had well-differentiated tumors. Lymphocytes from subjects with a past history of IM or normal donors with significant anti-EBV antibody activity may be sensitized to EBV and/or viral-associated antigens (4,11,12). TF or other lymphokines from such subjects might have the potential to program and/or recruit uncommitted lymphocytes to react against EBV and/or viral antigens, and thus may serve as an appropriate source of TF for immunotherapy of NPC.

In previous studies, TF from EBV-positive donors converted the leukocytes of two of four patients with NPC to a reactive state against an antigen extract of NPC as measured by peripheral leukocyte migration inhibition in vitro (11). TF immunotherapy of two patients with NPC was associated with apparent slowing of tumor growth coincident with marked lymphocytic infiltration of the tumor and reconstitution of

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delayed cutaneous hypersensitivity to PPD and *Trichophyton* (4,12). As a result of our in vitro studies of TF (11) and our limited clinical experience with TF immunotherapy (4,12), a randomized, double-blind, clinical trial was initiated in Hong Kong in 1974. Patients with NPC were treated with conventional radiotherapy; one-half of the group also received dialyzable TF obtained from normal adults with a history of IM and/or with an antiviral capsid antigen (VCA) antibody titer of 1:64 or greater. The results of that trial are the subject of this report.

MATERIALS AND METHODS

From July 1974 to May 1977, 100 patients with stage III NPC, as defined by the criteria of Ho (21), were entered in the study. All of the patients received radical radiotherapy at the M. and H. D. Institute of Radiology and Oncology, Queen Elizabeth Hospital, Hong Kong, under the supervision of J. H. C. Ho. A definitive histologic diagnosis of NPC was made on all patients.

Criteria of Eligibility

Men and women between 20 and 69 years of age were eligible for the study. The patients had stage III NPC as defined by the criteria of Ho (21), which classified the disease into five clinical stages. Stage III disease includes local spread of the disease beyond the nasal fossa, oropharynx, or adjacent muscles or nerves below the base of the skull (T3) and/or involvement of lymph nodes in the lower cervical or N2 level irrespective of laterality or mobility of the involved nodes. All patients had indirect examination of the nasopharynx and larynx, chest x-ray, and liver function tests. Other criteria were that the patient had not received a course of chemotherapy and would probably be able to attend the Queen Elizabeth Hospital for follow-up for at least 2 years.

Radiation Therapy

Radiation therapy Plans A, B, or D are used for all patients (22). In Plan A, split fields are employed throughout, to deliver 420 cGy (4.5-meV photons, mean tumor dose per fraction) in four fractions per week to a total dose of 5040 cGy. In Plan B, the dose is 250 cGy/fraction in four fractions per week to a total dose of 6000 cGy to the nasopharyngeal region; the dose to the neck is 560 cGy (4.5-meV photons) at the 90% isodose level weekly × 7 through an anterior cervical field, with a median shield for the larynx and spinal cord. The supraclavicular fossae (SCF) are also shielded when the last fraction is given. Residual enlarged nodes are given two booster doses of 10- or 15-meV electrons of 375 cGy at the 90% isodose level/dose 3 days apart. In Plan D, two parallel opposed lateral fields covering en bloc the nasopharyngeal region and the neck above the SCF are used to deliver a 200-cGy central dose/fraction in five fractions per week to a total dose of 4000 cGy; then split fields are used, as in Plan B, to deliver another 2000 cGy in eight fractions over 2 weeks. Thus, the total dose to the nasopharyngeal region is 6000 cGy over 6 weeks; the dose to the cervical nodes above the SCF consists of 4000 cGy over 4 weeks plus 560 cGy weekly × 2; and the dose to the SCF is 560 cGy weekly for 6 weeks.

Plan A is employed when the primary tumor is apparently confined to the nasopharynx and the enlarged upper cervical nodes are not situated at too high a position to be wholly covered by the anterior cervical field. Plan B is employed when the posterior part of the nasal fossa is involved. Plan D is employed when there is involvement of the pharynx below the nasopharynx or the upper cervical nodes are too large or too high up to be entirely covered by the anterior cervical field.

Selection of TF Donors

Normal young adults with a history of IM supported by positive serology (Paul Bunnell or Mono spot test) 3 months to 6 years previously were recruited in Winnipeg to serve as TF donors. In addition, normal blood donors were screened through Red Cross blood donor clinics for serum anti-VCA antibody activity using the HRIK subline of Burkitt lymphoma cells in the standard immunofluorescent technique of Henle and Henle (23); those patients with an anti-VCA antibody titer of 1:64 or greater were also used as TF donors. All patients with a past history of hepatitis, malaria, or syphilis were excluded. Donors were screened for hepatitis B antigen and positive reactors were rejected.

Preparation of TF

TF was prepared using a modification of the technique of Lawrence and Al-Askari (24). Approximately 450–500 ml of blood was collected in a sterile Fenwal (JH-1E) plastic bag (Baxter Laboratories, Malton, Ontario, Canada) containing heparin, passed through a Fenwal leukofilter (LP-1 Leuko-Pak) to remove granulocytes, and centrifuged at 2300 rpm for 5 minutes in a Sorvall RC-3 centrifuge. The plasma and mononuclear-rich buffy coat were removed with a Travenol extractor into AE-7 Plasma Transfer Set (Baxter Laboratories) and centrifuged at 5000 rpm for 10 minutes. The supernatant plasma was removed; the buffy coat in a final volume of 5–10 ml was freeze-thawed seven times and ultrafiltered through a PM-10 (76 mm) Diaflo membrane (Amicon Corporation, Lexington, MA). Each 3–5 ml of clear, colorless ultrafiltrate, which contained

compounds of < 10,000 in molecular weight and represented 1 donor unit of approximately 0.75–1.5 × 10^9 mononuclear cells or 2.5–5 × 10^9 leukocyte equivalents, was filtered through a Millipore membrane (Millipore Corporation, Bedford, MA) and stored at -20°C. The ultrafiltrate had a maximal absorbance at 260 nm, and the presence of ribose was determined by the orcinol reaction as described previously (25).

For the trial, TF was prepared in batches, in which buffy coat preparations from 12 to 24 donors were pooled and TF was extracted. Patients were treated sequentially with different batches; however, with few exceptions, each patient was treated with the same batch of TF for the entire therapeutic course.

Randomization

Allocation to the control or TF treatment group was made as soon as the patient was selected for the study, immediately following the administration of a complete course of radiotherapy. A patient register was supplied to the Queen Elizabeth Hospital in Hong Kong, where all patients were treated, and to investigators at the Manitoba Institute of Cell Biology, Winnipeg, Canada, where the control and TF vials were prepared. Randomization to control and TF groups was determined in advance of the study by the data coordinating center at the Epidemiology Unit of the National Cancer Institute of Canada, Toronto. The serial number for each patient was automatically obtained by entering the patient's name and age in the next available line in the register; it was forwarded promptly from Hong Kong to Winnipeg and Toronto. At the same time the patient's complete supply of treatment, which consisted of 30 vials labeled only with a serial number, was identified.

Conduct of the Study

The study was conducted "double-blind," with neither the patient nor the clinician aware if the patient was receiving saline or TF. All patients were in apparent complete remission following radiation therapy. Treatment was started on the last day of radiotherapy; it consisted of 1 ampule of saline or 1 donor unit of TF im once weekly for 3 months, then once monthly for an additional 18 months. No toxicity was encountered from the administration of TF. Any patient having a clinical relapse was declared a treatment failure and could then be treated either with further radiotherapy and/or chemotherapy, at the discretion of the attending physician. Despite clinical relapse the immunotherapy program (with TF or saline) was continued to completion.

Follow-up

All patients were followed at 3-month intervals for the first 2 years and then at 3–6-month intervals thereafter. All patients were followed for at least 5 years; four of the patients were lost to follow-up but this occurred after the 5-year observation period.

Statistical Methods

Survival curves were determined by the Kaplan-Meier procedure (26), with the significance of the difference of the two survival curves determined by the Wilcoxon rank-sum test (27). The influence of prognostic variables was evaluated using the log-rank test (28). The trial was planned on the basis of the sample size required to demonstrate a reduction in the uncontrolled tumor rate from 70% to 40% at a power of 0.8 and a significance level of 0.05.

RESULTS

The pretreatment status of the 100 patients with NPC allocated to either the control or TF treatment groups is presented in table 1. No significant difference was noted between the control and treatment groups with respect to age, sex, weight, performance status, or disease stage. There was no difference in hematologic findings prior to either radiotherapy or immunotherapy as shown in table 2.

A larger proportion of subjects in the control group received radiotherapy treatment Plan A; 45 control patients received Plan A compared to 39 patients in the TF treatment arm, as shown in table 3. Otherwise, the administration of radiotherapy to the nasopharynx, neck, and other sites was comparable in the two groups.

The survival curves of all patients with NPC treated with radiotherapy alone or with radiotherapy and TF are shown in figure 1. A trend appeared, suggesting that use of TF immunotherapy, if anything, might have a deleterious effect on survival; however, this difference was not statistically significant. The difference in survival of control and treated patients was reduced if the comparison was restricted to those patients receiving radiotherapy treatment Plan A (fig 2). This finding suggests that there was an inequality in randomization of patients receiving radiotherapy treatment Plans B and D between the control and TF groups. Of the control group, 45 patients received treatment Plan A, five received Plan B, and none received Plan D, whereas in the TF group, 39 patients received treatment Plan A, seven received Plan B, and four received Plan D (table 3). Although the number of patients treated with Plans B or D is too small to detect a statistically significant difference between the control and treated groups, the findings suggest that such patients may have a less favorable prognosis.

Similar findings were obtained for disease-free sur-

TABLE 1.—Allocation and pretreatment status of 100 patients with NPC*

Variable	Control		TF	
	Male	Female	Male	Female
Allocation				
Control	38	12	—	—
Treatment	—	—	40	10
Age (yrs)†	48.6 ± 1.9	53.1 ± 2.0	50.4 ± 1.4	45.2 ± 2.0
Weight (kg)†	47.0 ± 1.3	50.2 ± 2.6	51.1 ± 1.2	44.1 ± 3.2
Performance status‡				
0	1	2	4	0
1	31	9	34	8
2	5	1	2	2
3	1	0	0	0
T stage				
T3A	27	9	35	6
T3B	7	2	2	4
T3C	4	1	3	0
N stage				
N0	17	6	17	4
N1	8	5	10	4
N2	13	1	13	2

*Unless otherwise specified, values = No. of patients.

†Data are presented as mean ± SE.

‡Eastern Cooperative Oncology Group criteria: grade 0 = fully active patient, and grade 4 = completely disabled patient; none of the patients were grade 4.

vival whether all patients or only those treated with treatment Plan A were considered.

DISCUSSION

Although there are several reports in the literature of antitumor activity of TF (3-10,12), to our knowledge this is the only prospectively randomized, double-blind trial of TF immunotherapy of a malignant disease. The results of the trial clearly demonstrate that neither disease-free survival nor survival of patients with NPC treated with TF was significantly different than that of control patients receiving radiotherapy alone.

As with any negative study, there are several possible explanations of this result. Only patients with stage III NPC were included in the trial; it might be argued that the disease was too advanced to expect TF or any immunotherapeutic approach to be effective and that patients with less advanced disease might be more responsive. Patients with stage III disease were chosen for the trial because it was felt that the survival and relapse-free rates were sufficiently low to allow improvements in these rates to be recognized within a short period of follow-up if TF immunotherapy was beneficial. Furthermore, stage III is still locoregional disease. After a course of radiation therapy, the tumor load would be considerably reduced to allow TF to have a fair chance to show its effect.

Another reason for the negative result might involve the TF preparation. However, TF prepared with the

TABLE 2.—Hematologic status of 100 patients with NPC prior to radiotherapy and TF immunotherapy*

Variable	Control				TF			
	Male		Female		Male		Female	
	Preradiotherapy	Preimmunotherapy	Preradiotherapy	Preimmunotherapy	Preradiotherapy	Preimmunotherapy	Preradiotherapy	Preimmunotherapy
Hemoglobin (g/100 ml)	14.5 ± 0.3	13.6 ± 0.3	13.7 ± 0.4	13.1 ± 0.3	14.8 ± 0.2	13.8 ± 0.2	13.5 ± 0.3	13.3 ± 0.3
Count (× 10^3 cells/mm^3)								
Wbc	7.5 ± 0.4	5.3 ± 0.3	6.6 ± 0.6	4.6 ± 0.4	7.3 ± 0.4	6.0 ± 0.4	6.3 ± 0.7	4.6 ± 0.5
Granulocyte	68.9 ± 1.4	76.2 ± 1.5	68.3 ± 3.5	70.7 ± 1.9	70.7 ± 1.4	75.4 ± 1.5	68.6 ± 2.5	79.2 ± 2.8
Lymphocyte	22.8 ± 1.4	14.1 ± 1.3	24.8 ± 3.2	19.6 ± 1.9	21.5 ± 1.2	13.4 ± 1.0	25.8 ± 2.4	12.7 ± 1.7
Monocyte	5.2 ± 0.4	5.6 ± 0.5	4.8 ± 0.6	5.4 ± 0.7	4.8 ± 0.5	6.3 ± 0.6	4.1 ± 0.5	4.1 ± 0.5
Eosinophil	2.6 ± 0.5	4.1 ± 0.5	2.2 ± 0.6	4.3 ± 0.9	3.0 ± 0.4	5.1 ± 0.8	2.3 ± 0.8	3.9 ± 1.2
Platelet	197.2 ± 12.9	176.4 ± 11.4	189.0 ± 20.6	168.0 ± 19.0	181.0 ± 9.2	171.9 ± 8.7	204.4 ± 21 9	189.5 ± 18.4

*Values = mean ± SD.

TABLE 3.—Radiotherapy treatment plans for 100 patients with NPC treated with either radiotherapy alone or radiotherapy and TF*

Variable	Control		TF	
	Male	Female	Male	Female
Treatment plan				
A	35	10	31	8
B	3	2	6	1
D	0	0	3	1
Radiotherapy, nasopharyngeal region				
Yes	38	12	40	10
No	0	0	0	0
Radiotherapy, neck				
Yes	38	12	40	10
No	0	0	0	0
Radiotherapy, other				
Yes	7	0	12	1
No	31	12	28	9

* Values = No. of patients.

same methods was previously shown to be active in vivo by conversion of delayed cutaneous hypersensitivity reactions, induction of lymphocytic infiltration in the tumor, and apparent tumor regression (12); it was shown to be active in vitro by transfer of cellular immunity against NPC antigens in two of four patients with NPC in relapse and in seven of nine EBV-negative normal subjects (11). Even if the TF preparation was active, the dose of TF employed might have been inadequate and a more intensive dose schedule might have been more successful.

Another important lesson to be learned from this study, which has spanned 10 years and has involved three medical centers, is that carefully conducted clinical trials are extremely time-consuming, irrespective of whether the end result is positive or negative. Thus, it is of utmost importance to take great pains in the design of such trials and to have fairly persuasive data generated from preliminary or pilot studies to assist in the decision of whether to commit time and resources for a more complete formal trial. Despite such safeguards, the clinical trial might fail to support the hy-

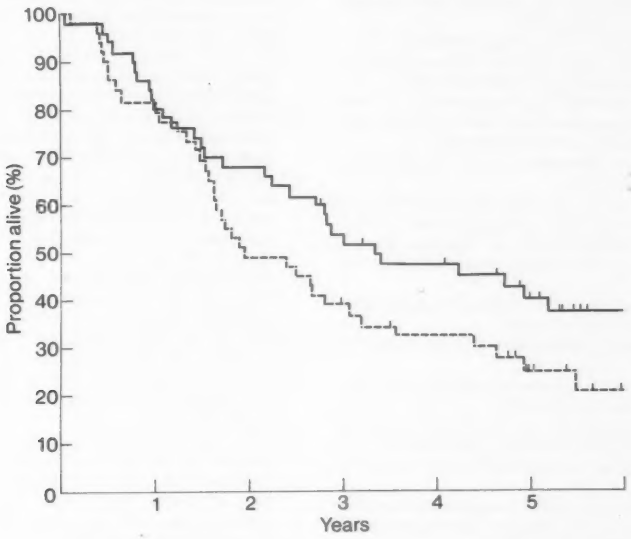

FIGURE 1.—Survival curves of all patients in the control group (———) and in the TF-treated group (- - - - - - - -) using the method of Kaplan and Meier.

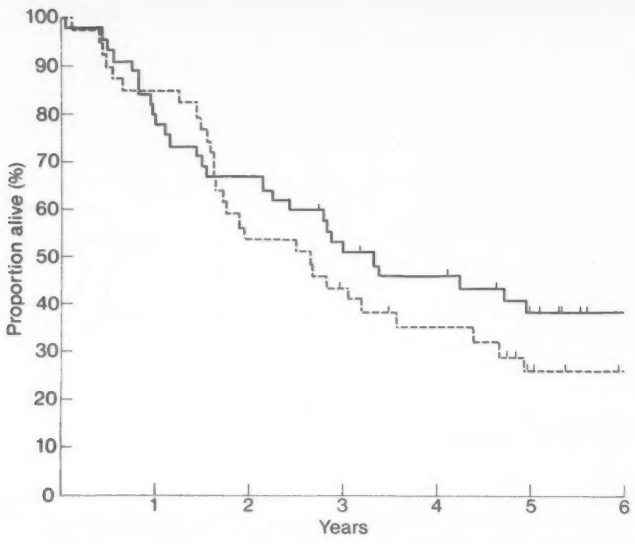

FIGURE 2.—Survival curves of all patients receiving radiotherapy treatment Plan A as described in the text. Control group (———); TF-treated group (----------).

pothesis suggested by the pilot study, as was our experience with this study.

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Alternation of Chemotherapy and Radiotherapy in Cancer Management. I. Summary of the Division of Cancer Treatment Workshop[1,2]

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The underlying therapeutic strategy for the clinical utilization of chemotherapy has evolved from the necessity of using large doses at intervals of 3–4 weeks to allow primarily for gastrointestinal tract and bone marrow recovery. The underlying therapeutic strategy for the clinical utilization of radiotherapy has evolved from the necessity of using small daily radiation doses to avoid major damage to the normal tissue surrounding the tumor. Further experimental and clinical studies are needed to better utilize these two primary treatment modalities in tumors known to be responsive to both chemotherapy and radiotherapy. Improvements in the use of well-established chemotherapeutic agents by more effectively combining them with radiotherapy can be brought to clinical use much faster and with greater efficiency than can new agents. New therapeutic approaches to improving cancer treatment will most likely be coordinated with chemotherapy or radiotherapy alone or combined. This underscores the need to learn how to optimally use these two primary therapeutic modalities to realize maximum effectiveness of new and future therapeutic methods.

A workshop was held at the National Cancer Institute on January 3, 1984, to discuss specific clinical and experimental studies directed toward a better understanding of the alternation of chemotherapy and radiotherapy in cancer management. The workshop was confined to a discussion of the relationship among three areas under investigation which hold considerable promise with regard to the more effective utilization of these two primary means of cancer management. The first area discussed was the superiority of alternating chemotherapy and radiotherapy in experimental studies at the Division of Radiobiology and Biophysics, University of Virginia School of Medicine, over conventional methods of utilizing these modalities. This was a brief summary of two reports from a previous series of papers (1,2). A more extensive discussion of this area and its relationship to clinical studies is included in a later paper of this series (3).

The second area, discussed by Dr. Goldie of the Cancer Control Agency of British Columbia, was the strategy of alternating the treatment regimen at every cycle to minimize the risk of developing doubly resistant tumor cells. The mathematical model developed by Goldie et al (4–6) relates the drug sensitivity of a tumor to its own spontaneous mutation rate toward phenotypic drug resistance. Their basic assumption is that tumors are curable by chemotherapy if no permanently resistant cell lines are present. However, curability diminishes rapidly with the appearance of a single resistant line if only one therapy is available or with the appearance of a doubly resistant line if two equally effective therapies are available.

Dr. Goldie discussed the preliminary results of mathematical modeling of the experimental studies at the Division of Radiobiology and Biophysics, University of Virginia School of Medicine. He and his co-workers were able to simulate the effects of these experimental results alternating chemotherapy and radiotherapy in the theoretical model. One of the most important ramifications of the theoretical fit of the experimental results is that large numbers of hypothetical experiments can be carried out. It will be possible to predict the outcome for other schedules of treatment so that only the more promising protocols need be subjected to experimental verification. The ability of the model to predict the outcome of completed experimental results will be covered in the second paper of this series (7).

Little et al (8) and Weichselbaum et al (9) of the Radiobiology Laboratory, Harvard School of Public Health, and the Joint Center for Radiation Therapy, Harvard

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Medical School, have suggested that repair of potentially lethal damage may correlate with clinical radiocurability (8,9). One of the most significant implications of these studies relates to the possibility that the potential for repair in different tumors may be the critical factor in the success or failure of radiotherapy. These results imply that results of experimental and clinical studies determining the effects of the initial therapeutic insult may be inadequate in the prediction of the outcome of multiple treatment schedules over a number of weeks. Several classes of agents have been described, which appear to inhibit or suppress this recovery phenomenon. However, little is known about the effects of chemotherapeutic agents on recovery of tumor cells from radiation-induced damage. The potential for therapeutic exploitation of these differences in repair of potentially lethal damage was discussed by Dr. John Little of the Harvard School of Public Health in the third part of the workshop.

Some pilot clinical studies are under way and others are being developed which will test the concepts that are evolving from these experimental and theoretical studies. Eight different clinical groups in this country and in Europe are beginning or plan to begin to assess the clinical effectiveness of alternating chemotherapy and radiotherapy in a number of types of tumors (3). The excellent therapeutic results and host tolerance in these pilot clinical studies with response rates and median survival in excess of those for more conventional utilization of chemotherapy and radiotherapy warrant further and more extensive evaluation in controlled clinical trials. Results for Hodgkin's disease [Hoppe et al (10) and Halberg et al (11)] and small [Tubiana et al (12,13)] and non-small cell [Arcangeli et al (14,15)] lung cancer in three different cancer treatment centers underscore the potential for improvement of cancer treatment in these and other tumor types by more effectively utilizing these two primary treatment modalities. Results of both the clinical and experimental studies also underscore the validity of these concepts for use in future studies in tumors which are responsive to both chemotherapy and radiotherapy.

Alternating Chemotherapy and Radiotherapy

One of the most clinically relevant concepts that has evolved from experimental studies at the Division of Radiobiology and Biophysics, University of Virginia School of Medicine, is the superiority of alternating cyclophosphamide (CP) and radiotherapy in three courses of induction therapy (16). A cure rate of ≥ 50% was obtained in experimental solid tumors using this protocol, with acceptable toxicity to the host, as opposed to no cures from three or more courses of either modality alone at total doses that exceeded the permissible levels of damage to the host. The alternate use of chemotherapy and radiotherapy allows twice the recovery time from each modality and results in reductions in toxicity to within clinically acceptable limits for both modalities without loss of tumor control and cure rates. These concepts can be tested clinically. They offer the possibility of major improvements in cancer management in some tumor types if chemotherapy can be alternated with radiotherapy clinically, as has been successfully carried out in experimental solid tumor systems.

No cures were obtained following single doses of 50–250 mg/kg (0.3–1.5 g/m^2) of CP in the experimental solid tumor 3924A in the ACI rat. There were no cures with sequential doses of 150 mg/kg of CP every 11 days for two, three, four, and five courses (total doses, 300–750 mg/kg of CP). Three courses of a total dose of 150 mg/kg of CP on Days 0, 11, and 22 are optimal with regard to tumor growth delay and median survival. Rats require 10–12 days for recovery of bone marrow cellularity from single (LD10) doses of 5-FU, doxorubicin, and CP. This is approximately one-half of the bone marrow recovery time in man of 17–21 days for comparable doses of cancer chemotherapeutic agents (16).

Multiple daily fractionation of radiation (hyperfractionated radiation) is now being investigated in experimental and clinical studies (17).[7] The rationale for use of multiple fractions per day (MFD) radiation schedules is that normal tissue recovery is greater than tumor recovery (18–22). Large amounts of radiation (1500 rads) can be delivered by MFD schedules over short periods of time (1–2 days), with acceptable normal tissue toxicity. The use of MFD schedules (7 or 11 days apart) in 3924A has given tumor control equivalent to daily radiation fractionation schedules, with comparable total radiation doses for the two treatment schedules (23). More importantly, in the experimental system, MFD schedules but not daily schedules, when combined with CP, give 50% cure rates at total radiation doses permitted by normal tissue tolerance. These alternative fractionated schedules offer, therefore, the potential for improving the effectiveness of the interaction of radiotherapy and radiosensitizers, radioprotectors, and hyperthermia in addition to chemotherapy.

The schedules for MFD were devised by increasing the number of 250-rad fractions over a 2-day period (2,16,23). These 2-day schedules were given at 11-day intervals for three courses on Days 0 and 1, 11 and 12, and 22 and 23. CP (150 mg/kg) was given 1 day after the three radiation courses on Days 2, 13, and 24. The total radiation dose ranged from 3000 to 9000 rads for radiation alone and from 750 to 7500 rads for radiation combined with CP. No cures were obtained with daily radiation totaling 11,250 rads or with MFD radiation totaling 9000 rads. The total dose for a 50% cure rate was reduced from the clinically unacceptable dose of 11,250 rads for daily fractions with CP to 6000 rads for MFD

[7]Radiotherapy Workshop on Time-Dose Relationships was held at the Division of Cancer Treatment in 1981. This workshop was summarized by E. Gilbert and J. Cox and published in the *DCT Bulletin*, June 1981, pp 4–5.

TABLE 1.—Radiation dose required for tumor growth delay and 50% tumor cures

	Fractionation schedules (rads)			
	Radiation alone		Radiation + CP	
	MFD	Daily	MFD	Daily
Growth delay of 100 days	8400	9200	4200	5600
Tumor cures = 50%	> 9000	> 11,250	6000	11,250

schedules with CP, which is within the clinically acceptable range (table 1).

The total radiation dose needed to give a growth delay of 100 days was 9200 rads for daily radiation given alone and 8400 rads for MFD given alone. The addition of CP to the daily fractionation schedules reduced the total dose needed to give a growth delay of 100 days by 39% (5600 vs 9200 rads). The addition of CP to the MFD schedules reduced the total dose needed to give a growth delay of 100 days by 50% (4200 vs 8400 rads).

A second series of experiments with MFD radiation schedules were carried out in which the time between each of the three 2-day radiation courses and CP was increased from 1 to 7 days (table 2). In the second series of experiments, radiation was given on Days 0 and 1, 14 and 15, and 28 and 29, and this was alternated with three single doses of CP given 1 week after each of the three courses of radiation, on Days 7, 21, and 35 (1). Giving CP 7 days after the initiation of the 2-day radiation schedules on Days 7, 21, and 35 increased the total time of delivery of the three courses to 35 days from 24 days in the previous series. This gave a 14-day recovery period for each modality as opposed to a recovery period of 11 days in the previous series. The reduction in mean tumor volume was comparable in both series, with the same total radiation dose of 7500 rads. A cure rate of 70% was achieved after 7500 rads plus CP 7 days after radiation, compared to a cure rate of 60% in the previous series giving CP 1 day after each of the three courses of radiation.

Increasing the total radiation dose from 6000 to 7500 rads in the series given CP 1 day after each of three courses of radiation resulted in an increase in total tumor cure rates from 50% to 60%. The tumor cure rate in the series given CP 7 days after radiation increased from 10% to 70% when the total radiation dose was increased from 6000 to 7500 rads.

In other experiments, the three 150-mg/kg doses of CP were given at 11-day intervals before any radiation was started and the radiation was then given at the rate of 250 rads/day over a 30-day period, for a total dose of 7500 rads. This schedule was reversed in another experiment, first giving 7500 rads over a 30-day period as 250-rad/day fractions and following with three courses of 150 mg/kg of CP at 11-day intervals. No cures were obtained for either schedule. These data are in contrast to the 70% cure rate in the experiments above in which a course of one modality, MFD radiation, was alternated with the other modality for three courses. The same total dose of 7500 rads and three courses of CP of 150 mg/kg at 11-day intervals was given in all of these comparisons (W. B. Looney and H. A. Hopkins, unpublished results).

The maximum skin response for the 6000 rads plus CP group (1.0 ± 0.09 arbitrary units) was smaller than that for the 7500 rads plus CP group (1.8 ± 0.09 arbitrary units). Recovery of the skin to a reaction of ≤ 0.5 occurred on Day 160 for the 6000-rad group and on Day 230 for the 7500-rad group. Increasing the total radiation dose from 6000 to 7500 rads increased the magnitude of the acute skin reaction as well as the duration of recovery. However, the skin reactions for both the 6000 and 7500 rads were well below the unacceptable upper limits of skin reaction of 2.0 arbitrary units defined by Fowler et al (24). Our data and the results of other investigators indicate that radiation combined with CP results in no increase in skin reaction compared to radiation alone (25–27).

The separation of the delivery of the two modalities by ≥ 1 week provides extended periods of time for normal tissue recovery. Separation of the delivery of radiation and CP by 7 days results in the delivery of CP every 14 days when the two modalities are alternated during successive courses of treatment. The recovery from CP in the rat occurs in 5–6 days in the gastrointestinal tract and in 10–11 days in the bone marrow. Excessive clinical toxicity from the simultaneous delivery of the two modalities has been one of the major drawbacks of combined therapy in the past. Results of these experimental studies, therefore, suggest that one of the major deterrents to the effective utilization of these two primary means of cancer management may be avoided by temporal separation of delivery while maintaining therapeutic efficiency.

The alternate utilization of the two modalities which results in increased effectiveness may be related to well-defined periods of accelerated growth, which occur following administration of either modality. Tumor growth rate changes following CP in the dose range of 50–250 mg/kg have been determined. Accelerated tumor growth was observed between 10 and 18 days after CP administration. Time of maximum tumor growth (T_{max}) was preceded by increased tumor cell proliferation which was preceded by an increased rate of DNA synthesis. Biochemical studies demonstrating increased cellular proliferation confirm accelerated tumor growth following treatment. Radiation given during this period of ac-

TABLE 2.—Total radiation dose required for tumor cures and local tumor control following MFD radiation schedules alone or in combination with CP with either a 1- or 7-day interval between modalities

	MFD (rads)		
		Radiation + CP	
	Radiation alone	1 day*	7 days*
Volume (Day 50)† = 250 mm^3	8300	3100	< 4500
Growth delay of 100 days‡	8400	4200	< 4500
Tumor cures = 50%	> 9000	6000	> 6000 but < 7500

* Interval between radiation and CP.

†Volume 50 days after beginning treatment. Tumor volume taken at initiation of treatment = $V_0 \simeq 250\ mm^3$.

‡Growth delay is taken at the time to reach 8 times initial volume.

celerated growth following CP treatment has the advantage of irradiating a more actively proliferating population of cells as well as allowing the host to recover from acute effects of the drug. Similar periods of accelerated tumor growth occur following radiation; the same advantage would exist with regard to CP given after radiation, as was done in these experiments (16).

Studies on tumor volume changes in patients with lung cancer have shown that a tumor volume nadir occurs 5-10 days after a single dose of radiation (range, 300-700 rads) and that an accelerated tumor volume increase then occurs over an 8- to 40-day period. A similar time frame for response also occurs in dogs. The lung tumor volume nadir was 5-6 days and the period of accelerated tumor volume increase was 5-15 days after a single dose of radiation in the 180-300-rad range. In the mouse, the lung tumor volume nadir was shorter, about 2 days, and the time of accelerated tumor volume increase was less, about 3 days, than for man, dogs, and rats (28-31). Thus, results indicate that perturbations in tumor growth rates in rats following radiotherapy and chemotherapy have comparable counterparts in both man and dogs.

Minimizing the Risk of Developing Doubly Resistant Tumor Cells

One of the most promising aspects of this approach was discussed at the workshop: there is little clinical evidence to date to suggest a buildup of resistance to radiotherapy alone or development of cross-resistance between chemotherapy and radiotherapy. The evidence suggests rather that there may be a cross-sensitivity between the two modalities.

Using a computer-based model of drug resistance, Goldie and his associates at the Cancer Control Agency of British Columbia simulated the effects of combined chemotherapy and radiation therapy treatments, specifically looking at the question of the appropriate timing for the use of radiation treatment. The basic assumptions involved in the simulation were as have been described in previous reports (4-7). In the hypothetical situation being examined, the limiting assumptions included (*a*) that the tumor could not be eradicated by either chemotherapy or radiation therapy alone and (*b*) that the function of the radiation therapy was essentially nondiscriminant; ie, it affected all proliferating cells within the tumor whether they were drug-sensitive or -resistant.

When chemotherapy was administered first there was an initial reduction in overall size of the tumor with respect to the sensitive and singly resistant compartments of clonogenic cells. Since there already existed a significant doubly resistant component, these are unaffected by alternating chemotherapy and as a result the tumor after a number of treatment cycles began to regrow in the face of continuing treatment. Application of the nondiscriminant radiation therapy at this time produced further reduction in size of the tumor but did not eradicate the doubly resistant compartment. Since there was still a viable clonogenic cell fraction surviving, the tumor would continue to grow and would also be resistant to further application of chemotherapy at this time. This constituted a failing therapeutic strategy.

In the second simulation the initial conditions of the tumor were the same as previously assumed, as were the log-kill parameters of the chemotherapy and the radiation therapy. In the second set of circumstances, however, the radiation therapy was given first. This resulted in significant tumor regression and also in the extinction of the doubly resistant compartment by a process of "dilution" of these cells from the bulk of the tumor. Application of the chemotherapy now resulted in eradication of the residual tumor after a few cycles of chemotherapy.

It is emphasized that no quantitative change in therapeutic effect was given in terms of the two different simulations. The only matter that was changed was the timing of the radiation. When given at the start it allowed the tumor to be rendered curable by chemotherapy, whereas when the radiation was given in a "consolidating" mode it resulted in further regression of tumor but failed to produce eradication. Moreover, in the noncurative strategy the continued growth of doubly re-

sistant cells during the period of chemotherapy application would have permitted the possibility of metastases from these doubly resistant clones emerging during the course of therapy. Therefore, such a strategy would have resulted in failure to control the primary tumor and in peripheral drug-resistant disease as well.

This very simple model of combined therapy effects should be testable at both the clinical and experimental levels. A simple experiment that could be done would be to deliberately mix a known proportion of drug-sensitive and -resistant cells within the same radiation field. A dose of radiation sufficient on average to extinguish the resistant subpopulation but to leave behind a sensitive subpopulation could then be applied. Testing of the residual tumor by the appropriate bioassays should reveal whether or not the predicted effect in diluting out the resistant cells had occurred.

A selective effect of the radiation against the chemotherapy-resistant cells was not specifically assumed in this case. The data of Looney et al (1) are compatible with this interpretation. If such a situation prevails, however, it would still predict for the superiority of the initial as opposed to late application of radiation. Under these conditions, the effect of the radiation could be mimicked by presuming it to function as a non-cross-resistant alkylating agent type of modality. As has been published previously (4–6), this would predict for enhanced therapeutic effect.

The question of an additive or synergistic interaction between radiation and chemotherapy on basically the same cell population was not addressed in the simulation. The experimental data showing that the benefit of alternation is sustained up to several days postradiation would appear to argue against a pharmacological explanation for the enhanced therapeutic activity of radiation and chemotherapy, ie, effects on sublethal repair would be expected to be abolished by waiting several days before the application of the next cycle of treatment. Thus, the experimental data suggest either a dilution effect, as described above; a situation analogous to the use of two non-cross-resistant alkylating agents; or possibly a combination of both such effects.

Several components of this theory are readily amenable to testing at the experimental level and are also feasible for clinical evaluation as well. Experimental studies in progress should provide additional information on whether one modality selectively kills a subpopulation of tumor cells which are resistant or sensitive to the other modality (W. B. Looney and H. A. Hopkins, unpublished results). This study will begin to provide information on the possible development of cross-resistance and cross-sensitivity of alternating chemotherapy and radiotherapy during treatment. The experimental results of Looney et al (16) and theoretical predications of the Goldie and Coldman model (4–6) both indicate that alternation of three courses of chemotherapy and radiotherapy is superior to three courses of either modality alone for induction therapy.

Possible Relationship of Inhibiting the Repair of Potentially Lethal Damage to the Superiority of Alternating Chemotherapy and Radiotherapy

Little et al (8) and Weichselbaum (9) summarized recent work at the Radiobiology Laboratory, Harvard School of Public Health, and the Joint Center for Radiation Therapy, Harvard Medical School, which attempted to determine whether the differing curability by radiation therapy of certain types of human tumors might reflect differences in intrinsic cellular sensitivity to radiation. Cell lines were established from a number of human tumors and examined for sensitivity to the cytotoxic effects of x-irradiation in vitro. Tumor lines established from osteogenic sarcomas, glioblastoma, medulloblastoma, neuroblastoma, hypernephroma, melanoma, and breast carcinoma showed no systematic differences in survival curve parameters following irradiation with single doses of x-rays during exponential growth. Also, no systematic differences were observed in the ability of these various types of tumor cells to recover between split doses of irradiation.

On the other hand, significant differences were observed in their response when these tumor cell lines were irradiated in the density-inhibited, plateau phase of growth. Under these conditions, the rate of cellular proliferation is markedly reduced and a large fraction of the cells are held in G_1. Subculture to low density is a stimulus for these cells to initiate DNA synthesis and resume exponential growth. Previously, it was shown that holding such cells in this nonproliferative growth phase by delaying subculture for 6–24 hours after irradiation will considerably enhance their eventual survival. This is a type of repair of potentially lethal damage (PLDR).

When these same tumor lines were examined for their ability to carry out PLDR after irradiation of density-inhibited culture, marked differences were observed. One osteosarcoma and two melanoma cell lines were markedly proficient in PLDR (tenfold enhancement in survival under standardized conditions), whereas two breast carcinoma cell lines performed very little PLDR (1.5–2.0-fold survival enhancement). Several other tumors including another osteosarcoma, a neuroblastoma, and a hypernephroma showed an intermediate response (twofold to fourfold enhancement in survival under standardized conditions). These results were encouraging since, in contradistinction to the results in exponentially growing cells, they showed distinct differences in the cellular response of human tumor cells to x-irradiation which correlate in a general way with the clinical response (local control) of the parent tumors. It has long been known that malignant tumors contain a significant fraction of nonproliferating but potentially clonogenic cells, which

might well be the controlling cells in the clinical response to radiation therapy or chemotherapy.

To investigate the effect of this phenomenon during fractionated irradiation, two cell lines were chosen as representative of those which showed maximal and minimal capacity for PLDR. These were the C 143 human melanoma and the MCF-7 human breast carcinoma-derived cell lines. Density-inhibited cultures were exposed to four equal doses of radiation separated by either 2 or 24 hours. Subculture to assay for ultimate survival was carried out 24 hours after the last exposure. A comparison of the survival curves obtained under these conditions indicated a D_0 for the melanoma cells about three times higher than that for the breast carcinoma cells. The high capacity for PLDR among the melanoma cells gave a marked radioresistance to these cells following fractionated exposure to a largely nonproliferating cell population.

These results indicate that PLDR, which occurs in nonproliferating tumor cells, may be a significant determinant in their response to fractionated radiation exposure. Recently, several classes of agents have been described which appear to inhibit or suppress this recovery phenomenon. Little is known, however, about the effects of chemotherapeutic agents on the ability of tumor cells to recover from such radiation-induced damage. It is indeed possible that some of the interactions observed between chemotherapy and radiation in experimental animal systems might reflect an effect of chemical agents on the cells' ability to recover from radiation damage. This hypothesis can be readily tested in vitro by use of this human tumor cell model for measuring PLDR.

The information that has accumulated on established cancer chemotherapeutic agents with regard to mechanisms of actions, therapeutic effectiveness, and toxicity makes them attractive candidates as inhibitors of PLDR following radiation (32,33). The possibility of an agent that can both inhibit PLDR and effectively act on the tumor independently is an attractive approach to increasing the effectiveness of radiation. This appears to be a promising area for further studies with CP and other alkylating agents and with other classes of cancer chemotherapeutic agents.

DeVita (34,35) recently has summarized concepts relating to the development of resistance to chemotherapy. He concludes that it is obvious that more effective anticancer drugs are still required to maximally exploit our current knowledge in cancer chemotherapy, but it also seems likely that more can be achieved with current drugs. The complicated nature of the assumptions of the Goldie-Coldman model, when individual variance in specific tumors, drugs, and clinical circumstances are added, makes it likely, however, that these advantages will ultimately accrue only with the help of computer modeling in advance of treatment, to design idealized protocols or individualized treatments for certain diseases as suggested by Goldie and Coldman and Skipper.

Improvements in the use of well-established chemotherapeutic agents by more effectively combining them with radiotherapy can be brought to clinical use much faster and with greater efficiency than can new agents. New therapeutic approaches to improving cancer treatment will most likely be coordinated with either chemotherapy or radiotherapy or combined chemotherapy and radiotherapy. The promising therapeutic results of clinical studies in this country and in Europe underscore the validity of the concepts that have evolved from experimental studies as well as the potential for improvement of cancer management by more effectively utilizing chemotherapy alternated with radiotherapy. This underscores the need to learn how to optimally use these two primary therapeutic modalities to realize maximum effectiveness of new and future therapeutic methods.

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Hemolytic Anemia Associated With Antineoplastic Agents[1,2]

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Hemolytic anemia is an uncommon, but important, complication following the administration of antineoplastic agents. Three types of hemolytic anemia have been reported: microangiopathic hemolytic anemia, immune hemolytic anemia, and oxidative hemolysis. Physicians should be alert to the possibility of this adverse effect in any patient who has a sudden or unexplained drop in hemoglobin concentration while undergoing cancer chemotherapy. If the hemolytic anemia is not recognized and allowed to continue, the consequences may prove to be fatal. [Cancer Treat Rep 69:777–782, 1985]

Anemia is a frequent complication in patients with malignancy. Usually it is a chronic, hypoproliferative anemia, which has been designated the anemia of chronic disease. However, hemolytic anemia associated with hematologic neoplasms and solid tumors is well-recognized. Recently, it has become apparent that hemolytic anemia also may be due to the administration of some antineoplastic agents. We review herein the drugs that can cause such anemia and discuss the mechanisms producing it. A list of these drugs, plus the type of hemolysis with which they are associated, is as follows: *microangiopathic hemolytic anemia (MAHA)*—mitomycin or cisplatin, bleomycin, and vinca alkaloid in combination; *immune hemolytic anemia*—chlorambucil, triethylenemelamine, cyclophosphamide, melphalan, teniposide, methotrexate, elliptinium, and cisplatin; and *oxidative hemolysis*—doxorubicin [in association with glucose-6-phosphate dehydrogenase (G6PD) deficiency], pentostatin, and IMPY.

MICROANGIOPATHIC HEMOLYTIC ANEMIA

MAHA is a distinct type of hemolytic anemia characterized by rbc fragments in the peripheral blood occurring in association with small-vessel disease. Since the original description by Brain et al (1), the relationship between MAHA and disseminated cancer has been well-documented (2–4). However, MAHA secondary to cancer appears to be a rare phenomenon. Antman et al (3) could find only 55 cases of this association in a review of the literature. Most of the cancers where MAHA has occurred have been mucin-producing adenocarcinomas, with gastric carcinoma being the most common (3). In addition to an association with cancer, MAHA has been reported to occur in patients with thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), disseminated intravascular coagulation (DIC), vasculitis, malignant or pulmonary hypertension, and prosthetic heart valves (5). Recently, the MAHA syndrome also has been reported to be related to therapy with mitomycin. In fact, since the original characterization of this problem in 1980, at least 115 cases have been described (6–38).

The clinical manifestations of this mitomycin-related syndrome consist of a basic triad of MAHA, renal failure, and thrombocytopenia. The thrombocytopenia is not part of a generalized coagulopathy, because most patients with this entity have exhibited no clinical or laboratory evidence of DIC. Other less consistent features include rash, fever, hypertension, neurologic changes, pericarditis, pulmonary edema, interstitial pneumonitis, congestive heart failure, hematuria, and proteinuria (6–38). Although some patients have recovered, in most patients the renal impairment has progressed to renal failure and death, usually within a few months after the diagnosis of MAHA-renal failure was established.

The principal histologic findings in patients with this problem have been abnormalities in the renal vasculature. The predominant features have been fibrin deposition and endothelial proliferation in the glomeruli and

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small arterioles. In addition, glomerular basement membrane thickening, reduplication, and disruption plus glomerular infarcts and necrosis have all been reported (7,8,10,12-14,16-18,23,25,29,36,38). Similar histologic abnormalities such as nuclear degeneration and necrosis, thickening of basement membranes and capillary loops, intimal hyperplasia of arteries, and fibrin thrombi in arterioles have been described in patients with nephrotoxicity secondary to mitomycin (7,39-42). Some of these instances may represent unrecognized cases of mitomycin-induced MAHA associated with renal insufficiency. It is noteworthy that the renal histopathologic findings described in MAHA related to mitomycin are similar to those seen in idiopathic HUS and TTP (43).

The frequency of MAHA-renal failure associated with mitomycin has been variable. Of 281 patients treated with mitomycin plus 5-FU in one study (27), 24 (8.5%) developed a MAHA-renal failure syndrome. In addition, 32 other patients (11.4%) had evidence of renal insufficiency. Of importance is the fact that no patients among 130 placebo-treated patients had MAHA-renal failure and only four (3%) had renal insufficiency in this study (27). Hanna et al (7) observed renal impairment in 14 of 143 patients (9.8%) treated with mitomycin plus 5-FU, two of whom had evidence of MAHA. Death from renal failure occurred in nine of these 14 patients. Pavy et al (16) treated 94 patients with mitomycin for a variety of cancers. Four of these patients developed renal insufficiency, and two of them had MAHA with renal failure. Liu et al (39) reported renal toxicity in three of 32 patients who received mitomycin, but none of them had MAHA. The findings from these studies suggest that there are two types of mitomycin-related renal problems: (*a*) a mild to severe renal insufficiency without MAHA; and (*b*) renal failure with MAHA. It is apparent that not every patient with renal impairment induced by mitomycin develops the MAHA-renal failure syndrome. On the other hand, all cases of MAHA are accompanied by signs of renal insufficiency.

MAHA-renal failure syndrome associated with mitomycin appears in most patients 4-12 months after beginning mitomycin therapy, but Khojasteh et al (34) described a patient who developed a hemolytic anemia syndrome 10 days following the administration of only 5 mg of mitomycin. In some patients, MAHA and renal failure have been reported to occur months after discontinuation of the mitomycin (11,16,18,28,32). This syndrome is unpredictable and may develop at any time while the patient is receiving mitomycin and even some months after drug administration ceases.

The pathogenesis of mitomycin-related MAHA is uncertain. One explanation is that it is due to a direct toxic effect of mitomycin on the kidney, and supporting this hypothesis is the fact that this drug is nephrotoxic in man (7,39-42). Furthermore, histologic abnormalities of the kidney have been present in every case of MAHA-renal failure syndrome where tissue was available for review (7,8,10,12-14,16-18, 23,25,29,32,36,38).

An alternate explanation for this syndrome is that it is immune-complex-mediated. Zimmerman et al (20) have suggested that circulating immune complexes form after chemotherapy reduces the tumor burden. These soluble antigen-antibody complexes then cause local vascular injury that results in the deposition of platelets and fibrin and leads to thrombosis of the microvasculature. Cantrell et al (24) have also observed that the antibodies in the immune complexes produce significant immunofluorescence against gastric and colon carcinomas, but show no reactivity with mitomycin. Although circulating immune complexes have been reported in some patients with this syndrome (8,17,19,24,28), immune complexes have not been demonstrated in renal biopsies from any of the patients.

Another hypothesis is that this syndrome is due to the cancer itself. Against this possibility is the fact that some patients have had no detectable tumor at autopsy and many patients were being treated with adjuvant chemotherapy after surgical removal of the primary cancer (8,10,13,16,23,25,27,30-32,35,36). Moreover, if cancer or circulating immune complexes were a cause of this syndrome, then one would expect the disorder to occur frequently in association with other antineoplastic agents. This is not the case, and therefore neither hypothesis appears likely. Instead, we postulate that the primary pathogenic mechanism is through mitomycin-induced damage to the renal endothelium, which activates the coagulation system and leads to fibrin thrombi deposition in the microvasculature of the kidney and other organs.

In this context it is noteworthy that an abnormality of prostacyclin metabolism may be a causative factor in the pathogenesis of TTP. Furthermore, damage to the vascular endothelium may be associated with increased levels of Factor VIII : von Willebrand factor antigen (VIII:vWFAg), and abnormalities of plasma VIII:vWFAg multimers have recently been described in chronic relapsing TTP (44) and acute HUS (45). Thus, future investigations to elucidate the MAHA syndrome pathogenesis might include the following: (*a*) the relationship of mitomycin to prostacyclin metabolism; (*b*) the in vivo and in vitro effects of mitomycin on platelet aggregation and vascular endothelial cells; and (*c*) determination of plasma levels of VIII:vWF complex before and after the administration of mitomycin.

In general, the treatment of the MAHA syndrome using steroids, antiplatelet drugs, and rbc transfusions has not been effective. In fact, rbc transfusions have been associated with further deterioration of renal and cardiopulmonary function (10,15,17,20,23,25,35,37). It has been proposed that such transfusions may activate the clotting system which could initiate MAHA (46). Plasmapheresis in combination with several other therapeutic

modalities has been used in some patients (6,19,20,22,24). Improvement in the MAHA occurred in some patients, but the renal failure was usually progressive. As yet, a salutary effect of plasmapheresis on the course of the syndrome has not been substantiated.

Mitomycin has been in clinical use since its discovery in the late 1950s, and it has been marketed in the United States since 1974. Despite this long experience with mitomycin, MAHA was not recognized until 1980. The reason for this late appreciation of such toxicity is not known, but clinicians should be alert to the possibility of MAHA developing in any patient being treated with systemic mitomycin. Careful monitoring of renal function and hematologic status for early detection of such toxicity is warranted during treatment and for a few months after its discontinuation. At the earliest sign of such toxicity, mitomycin therapy should be stopped, since MAHA can sometimes be reversible (19,21).

In addition to mitomycin, a few cases of MAHA and renal failure have been associated with cisplatin-based combination chemotherapy (47,48). Cerebrovascular accidents (48,49), accelerated hypertension (50), and Raynaud's phenomena (51) have also been observed during treatment with cisplatin-containing regimens. It appears that such cisplatin regimens can damage the microvasculature in multiple sites and in some patients initiate MAHA and renal failure.

IMMUNE HEMOLYTIC ANEMIA

Well-characterized cases of immune hemolytic anemia secondary to cancer chemotherapeutic agents have rarely been reported. Although several studies in the 1960s suggested that alkylating agents could provoke immune hemolysis in some patients with neoplasms, the mechanism of the drug-induced hemolytic anemia was not well-discerned (52–54). In 1967, Eyster (55) described a patient who developed acute hemolytic anemia following the administration of oral melphalan. Studies demonstrated that the patient's serum caused in vitro agglutination of normal compatible erythrocytes in the presence of melphalan but not in its absence. Furthermore, it appeared that the antidrug antibody was of the IgG type and did not fix complement. Thus, the investigator suggested that the pathogenesis of the hemolysis was probably via a drug-hapten mechanism. Although a negative direct Coombs' test was observed in this patient, it may have been related to drug concentration, since an in vitro concentration 1000-fold greater than the estimated blood level was necessary to produce a positive antiglobulin reaction. Hemolysis secondary to melphalan appears to be a rare event, because no additional cases of hemolytic anemia associated with this drug have been published.

Cisplatin-induced hemolytic anemia has been reported by several investigators (56–58). Getaz et al (56) described two patients who developed a positive direct antiglobulin reaction (Coombs' test) after prolonged administration of cisplatin. However, evidence of overt hemolysis was present in only one patient. Direct antiglobulin testing in both patients was positive, with polyspecific antiserum to human globulin and monospecific anti-IgG but not to anti-C3d. These investigators suggested that an IgG antibody directed against membrane-bound cisplatin, rather than against any specific rbc antigen, was responsible for the hemolysis. This type of drug-induced hemolysis is analogous to one induced by penicillin and has been referred to as the drug adsorption mechanism (59).

In another study, Levi et al (57) reported two additional cases of hemolytic anemia occurring after the administration of cisplatin. Both of these cases, however, are complicated by the fact that each patient had received blood transfusions prior to the development of hemolysis. The investigators even suggested that the mechanism of hemolysis in the second patient could represent a transfusion reaction unrelated to cisplatin. Furthermore, both patients were being treated with combination chemotherapy. Thus, it is difficult to ascertain whether these cases represent genuine examples of hemolysis induced solely by cisplatin.

Nguyen et al (58) described a patient who developed a positive direct antiglobulin reaction 12 hours after administration of his second course of cisplatin. It is noteworthy that although the antiglobulin reaction remained positive after subsequent courses of cisplatin, the hemoglobin concentration remained stable. This may indicate that rbc sensitization induced by cisplatin may be asymptomatic and mild, and thus its presence does not necessarily preclude additional cisplatin treatment. This situation is comparable to the rbc sensitization seen with use of penicillin, where the presence of a positive Coombs' test does not necessarily mean the drug must be discontinued (59).

Since only a few cases of cisplatin-induced hemolysis are well-documented in the literature, the incidence of this drug reaction must be extremely low. Nevertheless, one should consider the possibility of an immune hemolytic anemia in any patient who has an unexplained drop in hemoglobin after treatment with this agent. Prospective studies would be useful to assess the frequency of positive antiglobulin tests and overt hemolytic anemia associated with cisplatin therapy. Such a trial would only require performing serial Coombs' tests on patients who are receiving this drug.

Woolley et al (60) have reported a case of acute hemolytic anemia following treatment with methotrexate. Studies by these investigators demonstrated that the hemolysis was due to an IgG3 antibody that reacted with erythrocytes in the presence of methotrexate. The antibody did not fix complement, but it sensitized rbcs to phagocytosis by macrophages. Although these investigators (60) concluded that the hemolysis was mediated by

an immune complex mechanism, they could not exclude a drug adsorption mechanism. The type of immune complex mechanism in this patient is different from the one associated with complement fixation. In the latter instance, activation of the complement cascade causes acute intravascular hemolysis while in the former, extravascular hemolysis occurs by means of the reticuloendothelial system.

One case of immune hemolytic anemia and renal failure secondary to teniposide has been reported (61,62). This patient developed acute hemolytic anemia and renal failure after a long period of teniposide treatment. The direct antiglobulin test was strongly positive because of in vivo coating of rbcs by IgG1. The in vitro reaction was complement-independent, and the indirect antiglobulin reaction was positive in the presence of the drug. The investigators (62) suggested that the pathogenesis of hemolysis was by an immune complex mechanism. To determine whether there was any occult antibody to teniposide in other patients, Habibi et al (62) studied 48 additional patients on this drug, but no other instances of such antibodies were found.

Acute hemolytic anemia has been reported to occur in association with the investigational drug elliptinium, which is a new class of antitumor agent currently undergoing clinical trial in the United States and Europe (63-65). In one prospective study of 146 patients undergoing treatment with this drug, ten patients (6.8%) developed hemolysis (64). This study also demonstrated that the hemolysis correlated with the schedule of administration, occurring more frequently in those patients treated weekly than in those treated every 2-4 weeks (64). It has been suggested that the pathogenesis of elliptinium-induced hemolysis is via an immune complex mechanism with IgM as the antibody (63,64). The presence of this IgM antibody indicates a high risk for the development of hemolysis (64). When hemolytic anemia occurs, treatment with elliptinium must be discontinued. The drug sponsor (Ives Laboratories) currently recommends performance of a test for antielliptinium antibody before each drug dose. If the antibody titer exceeds 1:32, treatment should be stopped.

With the exception of elliptinium, it appears that immune hemolytic anemia secondary to antitumor agents is a rare phenomenon. Only isolated cases of this problem have been observed with melphalan, methotrexate, cisplatin, and teniposide. Although anemia is common with prolonged administration of cisplatin, the major mechanism of this toxicity seems to be deficient rbc production (66) rather than hemolysis, either overt or latent.

OXIDATIVE HEMOLYSIS

It is well known that the rbc is susceptible to a host of metabolic insults associated with the administration of certain pharmacologic agents or their metabolites. Although several in vitro studies have been published describing interference with rbc metabolism due to exposure to cytotoxic drugs (67-69), only a few cases of oxidative hemolysis have been reported.

A case of oxidative hemolysis following the administration of doxorubicin in a patient with G6PD has been described (70). Following the initial dose of doxorubicin, the patient experienced a sudden drop in hemoglobin concentration accompanied by reticulocytosis, hemoglobinuria, and hemoglobinemia. In addition, Heinz bodies were demonstrated in the rbcs, and the direct and indirect antiglobulin reactions were negative. It has been shown that doxorubicin generates reactive oxygen compounds (71) and methemoglobin (67) in normal human rbcs in vitro. Moreover, doxorubicin poses oxidant stress to rbcs deficient in G6PD (72). Thus, patients with G6PD deficiency may be susceptible to hemolysis after receiving doxorubicin or related compounds.

Pentostatin, a potent inhibitor of adenosine deaminase, is undergoing clinical trial in leukemia and certain T-cell lymphomas. Acute hemolytic anemia has been observed following the administration of this agent (73,74). In one study of 17 patients with acute leukemia refractory to conventional chemotherapy, nine patients developed acute hemolysis after therapy with pentostatin (74). The hemolysis was acute in onset and developed during the first 10 days of the pentostatin administration (74). However, insufficient clinical information was available to determine whether the hemolysis was intravascular or extravascular and whether or not it was dose-related. The exact mechanism of the hemolytic anemia has not been defined, but there is evidence to suggest that it is due to a marked reduction in erythrocyte ATP levels (73).

Another investigational agent associated with acute hemolytic anemia is IMPY (75,76). This drug is a potent inhibitor of DNA synthesis, but does not inhibit RNA or protein synthesis. Acute intravascular hemolysis has been observed in a high percentage of patients after the administration of this drug, and this has led to death in one patient (75,76). There is evidence of generation of hydrogen peroxide in rbcs in vitro and evidence of oxidative stress by methemoglobin formation in vivo associated with this drug (76). In addition, a fall in rbc ATP levels has been observed in patients receiving this cytotoxic agent (76). Therefore, although the exact nature of the hemolysis is not clear, it appears that it may be due to oxidant stress. Investigational trials with this agent have now been closed because of this hemolysis toxicity and no meaningful activity in human cancers.

CONCLUSION

It is evident from the above discussion that hemolytic anemia may occur in association with some antineoplastic agents. By far the most common types of hemolytic

anemia are MAHA associated with mitomycin therapy and immune hemolytic anemia occurring from elliptinium. Unfortunately, in most cases the MAHA-renal failure syndrome is unpredictable and eventually leads to death. Less commonly, immune hemolytic anemia has been reported following the administration of a few other chemotherapeutic drugs and, rarely, oxidative hemolysis has been described. One should consider the possibility of drug-induced hemolytic anemia in any patient who has a sudden or unexplained drop in hemoglobin concentration while undergoing treatment with antineoplastic agents. If the hemolysis is not recognized and allowed to continue, it may prove to be fatal.

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Etoposide, Carmustine, Bleomycin, and Methotrexate With Leucovorin Rescue as Re-treatment for Unfavorable Non-Hodgkin's Lymphoma[1,2]

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Fifteen patients with unfavorable, non-Hodgkin's lymphoma refractory to front-line chemotherapy were treated with etoposide, carmustine, bleomycin, and methotrexate with leucovorin rescue. Four patients achieved complete response and two achieved partial response. The durations of the complete responses were 4, 12, 24, and 32 months, respectively. Three of the four complete responses occurred in five patients who failed to respond to initial combination chemotherapy. The major toxic effects were severe myelosuppression in eight of 52 courses and pulmonary fibrosis in two patients. This regimen is useful in inducing durable remissions in patients with refractory, unfavorable, non-Hodgkin's lymphomas. [Cancer Treat Rep 69:783–786, 1985]

Combination chemotherapy regimens can achieve complete response rates of 40%–70% in previously untreated patients with unfavorable non-Hodgkin's lymphomas (1–5). Although some patients are cured by front-line therapy, 30%–60% of patients who achieve complete remission eventually relapse. For the patients who fail to achieve complete remission or for those who relapse from complete remission, subsequent remissions with front-line therapy are uncommon and survival is short.

Approximately 30% of the patients with favorable non-Hodgkin's lymphoma develop a biologically aggressive disease with histologic conversion to high-grade lymphoma (Richter's syndrome) (6,7). Since many of these patients have received prior radiation therapy and chemotherapy, an effective re-treatment regimen is needed for this subpopulation.

We have combined four cytotoxic drugs, which lack cross-resistance with frequently used front-line chemotherapy agents, as a re-treatment regimen for patients with unfavorable, non-Hodgkin's lymphomas who fail to respond to or who relapse from complete remission after initial treatment. The salvage regimen includes etoposide (VP-16), carmustine (BCNU), bleomycin, and methotrexate with leucovorin rescue (VP-BBML). The results of this regimen are the basis for this report.

MATERIALS AND METHODS

Patients with advanced, unfavorable, non-Hodgkin's lymphoma who had failed to achieve complete response or who had relapsed following a complete response to front-line chemotherapy were entered in our study. All patients were evaluated at the Shands Teaching Hospital or at the Gainesville Veterans Administration Medical Center between June 1980 and May 1982. Patients who had previously received VP-16, BCNU, bleomycin, or methotrexate were excluded. No exclusions were made on the basis of performance status. The diagnostic biopsy preparations were reviewed by one of us (R. C. Braylan). For this study, unfavorable, non-Hodgkin's lymphomas included diffuse poorly differentiated lymphocytic; diffuse mixed lymphocytic-histiocytic; nodular histiocytic; and diffuse histiocytic according to the Rappaport classification (8). All patients had measurable disease on physical examination and/or plain radiographs and/or computed axial tomography. Bone marrow aspirate and biopsy and lymphangiograms were done when clinically indicated or to document extent of disease. Lesions on liver scan, bone radiographs, or bone scan were not considered measurable disease.

The chemotherapy regimen is as follows: VP-16 (100

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mg/m^2 iv on Days 1, 2, and 3); methotrexate (50 mg/m^2 iv on Day 1); leucovorin (5 mg orally every 6 hours for six doses beginning 24 hours after the methotrexate); bleomycin (10 mg/m^2 iv on Days 1, 8, 15, and 22); and BCNU (100 mg/m^2 iv on Day 1). The course was repeated every 4 weeks. Since VP-16, BCNU, and methotrexate have myelosuppressive effects, leucovorin was added to lessen the marrow toxicity due to methotrexate. The dosages of BCNU and etoposide were reduced by 50% of the initial, calculated dose if the granulocyte count nadirs were $< 600/mm^3$ or if the platelet count nadirs were $< 75{,}000/mm^3$. Subsequent doses were increased by 25% of the initial dose if the subsequent nadir counts were greater than the limits defined above. Bleomycin was discontinued after an accumulated dose of 320 mg/m^2 of body surface area or on clinical suspicion of pulmonary toxicity.

Complete remission (CR) was defined as the disappearance of all measurable disease and all disease-related symptoms for a period of 1 month; partial response (PR) was defined as a > 50% reduction in the sum of the products of perpendicular diameters of the measurable lesions and the absence of new lesions; stable disease was defined as a < 50% reduction in the sum of the products of perpendicular diameters of the measurable lesions and the absence of new lesions; and disease progression was defined as any increase in the size of the measurable lesions or the appearance of new lesions, regardless of the response at other sites of disease.

Patients were evaluated after every two cycles of therapy. Response was determined by physical examination, plain radiographs, and computed axial tomography. No repeat biopsies were obtained. Patients with progressive disease were removed from the study but were followed for survival. Patients with PR or stable disease continued to receive therapy until evidence of disease progression. Patients who achieved CR before or with Cycle 4 received a total of six cycles of chemotherapy. Patients who entered CR after Cycle 4 received two additional cycles after achieving CR. The Institutional Review Board approved this protocol. Written informed consent was obtained from each patient prior to entry in the study.

RESULTS

Fifteen patients were treated with this regimen. Patient characteristics are summarized in table 1. There were 11 males and four females. The median age was 55 years (range, 22–72). All patients had received combination chemotherapy before study entry. Five patients (Nos. 3, 6, 10, 12, and 15) had received radiation therapy. Bone marrow involvement was documented in four patients (Nos. 1, 5, 13, and 14). One patient (No. 1) had bulky disease, with a 6-cm retroperitoneal mass. Eight of the 15 patients had a Karnofsky performance status ⩽ 50% on entry in the study.

CRs were obtained in four patients. Two patients achieved PR and eight failed to respond (table 1). One patient (No. 14) died of infectious complications during granulocytopenia on Day 13 of the initial course of chemotherapy. He was evaluated for toxicity only. Response by performance status is also shown in table 1.

The CR durations were 4, 12, 24, and 32 months, respectively. One patient (No. 11) died with recurrent pulmonary emboli 4 months after achieving CR. At postmortem examination, there was also diffuse alveolar damage consistent with bleomycin toxicity, but there was no residual lymphoma. The median survival of the patients achieving CR was 23+ months (range, 10–36+); of patients achieving PR was 9.5 months (range, 7–12); and of the nonresponders was 4 months (range, 2–6). The minimum follow-up was 24 months from entry in the study.

The major toxic effect observed in our study was myelosuppression. Absolute granulocyte counts of $< 600/mm^3$ occurred in eight of 52 courses. Subsequent nadir counts allowed adjustment back to calculated dose in three of the five patients. Three patients had bacteremia during the granulocyte count nadir. One death due to *Escherichia coli* sepsis unresponsive to antibiotics occurred during the period of neutropenia following the first course (Patient 14). Subclinical pulmonary toxicity documented by pulmonary function tests occurred in Patient 6. Symptomatic pulmonary fibrosis occurred in Patient 8 and was a contributing factor in her death due to recurrent pulmonary emboli.

Minor toxic effects associated with this regimen included moderate nausea and vomiting, which occurred with approximately one-half of the delivered cycles. Transient erythema was noted in three patients after bleomycin administration, but resolved with continued therapy. One patient had elevation of serum transaminases to two times normal, with subsequent resolution of liver function tests on continued therapy. Various degrees of alopecia occurred in all patients. No cardiac, renal, or neurologic toxic effects were noted. No mucositis was observed.

DISCUSSION

With VP-BBML we report a CR rate of 29% and an overall (CR+PR) response rate of 43% in patients with advanced, unfavorable lymphoma that was refractory to front-line therapy. These response rates are especially encouraging because over half of our patients had a Karnofsky performance status ⩽ 50% on entering the study.

Patients with unfavorable, non-Hodgkin's lymphoma who fail to respond to front-line therapy or who relapse after CR are rarely salvaged with front-line chemotherapy regimens. Single-agent therapy with experimental drugs such as VP-16 (9–12), ifosfamide (13), amsa-

TABLE 1.—Patient characteristics and response to VP-BBML regimen

Patient No.	Age (yrs)	Sex	Histology*	Prior therapy†	Response to prior therapy‡	Duration of response (mos)	Performance status (%)	Response to present regimen‡	Duration of response (mos)	Survival (mos)
1	53	M	DHL	CHOP	NR	—	90	CR	32	36+
2	22	M	DHL	CHOP	NR	—	90	CR	24	26+
3	65	M	DHL§	CHOP + RT	NR	—	60	PR	—	12
4	35	M	DHL	CHOP	NR	—	50	NR	—	2
5	52	M	NHL	CHOP	NR	—	60	CR	12	19+
6	53	M	DHL¶	CHOP + RT	PR	29	40	PR	4	7
7	68	M	DHL	CHOP	PR	9	40	NR	—	6
8	65	F	DHL	CHOP	PR	1	60	NR	—	6
9	38	M	DHL	CHOP	PR	3	60	NR	—	3
10	62	F	DHL**	COP, CHOP, RT	CR	22	40	NR	—	5
11	66	F	DHL	CHOP	CR	1	60	CR	4	10
12	59	M	DHL	COPP + RT	CR	36	40	NR	—	5
13	62	F	DHL	C-MOPP	CR	3	50	NR	—	3
14	72	M	DHL	C-MOPP	CR	2	50	—	—	0.5
15	54	M	DHL	COP, CHOP, RT	CR	60	50	NR	—	2

* DHL = diffuse histiocytic lymphoma; NHL = nodular histiocytic lymphoma.

†CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; RT = radiation therapy; COP = cyclophosphamide, vincristine, and prednisone; COPP = cyclophosphamide, vincristine, procarbazine, and prednisone; and C-MOPP = cyclophosphamide, methotrexate, vincristine, procarbazine, and prednisone.

‡NR = no response.

§There was transformation from favorable to unfavorable histology (giant follicular lymphoma to DHL). Duration of initial histology was 11 yrs. RT was given prior to conversion.

¶There was transformation from favorable to unfavorable histology (nodular poorly differentiated lymphoma to DHL). Duration of initial histology was 6 yrs. RT was given prior to conversion.

** There was transformation from favorable to unfavorable histology (nodular poorly differentiated lymphoma to DHL). Duration of initial histology was 4 yrs. COP was given prior to conversion.

crine (14), or gallium nitrate (15) has yielded overall response rates of 0%–47% and CR rates of 0%–17%. Most of the CRs have been of brief duration.

More recently, several investigators have used combination chemotherapy as re-treatment for these patients. Cabanillas et al (16) reported an overall response rate of 67% (23 responses among 34 patients) and a CR rate of 38% (13 responses among 34 patients) with ifosfamide, methotrexate, and VP-16 in refractory patients with large cell lymphoma. At the time of the report, three patients had remained in continuous CR for > 2 years. The durable CRs in this study and in ours suggest that some patients with presumed refractory non-Hodgkin's lymphoma may be effectively re-treated with combinations of drugs which are non-cross-reacting with the induction regimen. Administration of the methotrexate at higher doses and/or the more frequent intervals as reported in other regimens (5,16,17) may improve the response rate.

The toxicity associated with our regimens was acceptable, especially in light of the previous exposure to multiple cytotoxic drugs and the generally poor performance status of our patients. Reversible myelotoxicity was the major side effect associated with this regimen. Fever and granulocytopenia required aggressive management with antibiotics and supportive care, as previously reported (18). Bleomycin-induced pulmonary toxicity was also observed in two patients, despite modest cumulative doses. However, our incidence of pulmonary toxicity is similar to that of other published series (19). Our regimen can be given entirely on an outpatient basis, thus allowing patients to spend relatively little time in the hospital.

Since a major aim of our study was to determine the effect of our regimen on biologically aggressive lymphomas, patients with favorable (indolent) lymphomas were excluded from the protocol. However, patients with indolent disease may undergo both histologic and biologic conversion to an unfavorable lymphoma (7). Many of these patients have been treated previously with multiple chemotherapies and/or radiotherapy. Resistance to cytotoxic drugs is a major concern within this subpopulation. Our series included three such patients (one achieving PR; two having no response). Unfortunately, this sample is too small to draw meaningful conclusions. Further study of re-treatment chemotherapy in a larger cohort is needed.

Patients who fail to attain CR with a doxorubicin-containing front-line regimen are thought to have primary resistant disease. However, the current regimen and other recent reports (16–19) demonstrate that durable CRs are attainable with regimens based on VP-16 and methotrexate. Aggressive combination chemotherapy with non-cross-reacting drugs is indicated for

these patients. The role of the current regimen in re-treatment after failure with mBACODAR PRO-MACE-MOPP [methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone (mBACODAR) or prednisone, methotrexate, doxorubicin, cyclophosphamide, VP-16, mechlorethamine, vincristine, and procarbazine (PRO-MACE-MOPP)] has not been studied.

A growing body of evidence suggests that some patients with unfavorable lymphomas can be effectively re-treated with VP-16/methotrexate-based regimens (16,17). This clinical experience implies that similar regimens may be incorporated into front-line strategies to improve both the induction rate and the duration of continuous CR for patients with unfavorable lymphomas. Clearly, properly designed randomized controlled trials will be necessary to test this hypothesis.

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Amsacrine in Refractory Acute Leukemia[1]

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Thirty-two patients with heavily pretreated, relapsed acute leukemia were treated with amsacrine (120 mg/m²/day × 5). The 32 patients received a total of 41 courses of therapy, and 31 patients were evaluable for response. There were no complete remissions and only one partial remission (3 months) in an adult patient with acute lymphoblastic leukemia. Toxic effects included myelosuppression (100% of the patients), hyperbilirubinemia (41%), nausea and vomiting (41%), stomatitis (9%), and cardiac dysrhythmia (3%). We conclude that amsacrine as a single agent is not a useful treatment for relapsed, heavily pretreated adult and pediatric acute leukemia. [Cancer Treat Rep 69:787–789, 1985]

Amsacrine is an acridine dye derivative which has been reported to have activity in patients with relapsed acute leukemia (1–11). Complete remission (CR) rates of 17%–28% have been observed in patients having acute nonlymphocytic leukemia (ANLL) who were treated with 450–1000 mg/m²/course. Similarly, CR rates of 9%–42% have been noted in patients with acute lymphocytic leukemia (ALL) who were given 225–1000 mg/m²/day per course. Since responses have been observed in many patients whose leukemia was thought to be resistant to anthracyclines, it has been suggested that amsacrine is the most effective therapeutic agent for heavily pretreated relapsed patients (1,2,6,7,9).

Based on these initial encouraging observations, a phase II study was begun in January 1981 at the Dana-Farber Cancer Institute using amsacrine as a single agent in adults and children with relapsed acute leukemia. Over a 22-month period, 32 patients were entered in the study. All of these patients had been heavily pretreated, and 27 had undergone previous attempts at reinduction with chemotherapy. The therapeutic results and toxic effects of amsacrine in this group of heavily pretreated patients are described in this report.

MATERIALS AND METHODS

Patients

Criteria for entry in this protocol included the diagnosis of ANLL, ALL, or the blast phase of chronic myeloid leukemia (CML-BC) resistant to standard therapies. Thirty-two patients were entered in this protocol, including seven pediatric patients (age range, 2–16 years old) and 25 adult patients (age range, 18–79 years old). Twenty patients had ANLL (three pediatric patients), nine had ALL (three pediatric patients), and two had CML-BC. One pediatric patient had dimorphic leukemia and has been diagnosed and treated for ANLL. The performance status of the patients in this study ranged from 70 to 100 (Karnofsky scale: 70 in one patient, 80 in 15, 90 in four, and 100 in 12). Seven patients were hospitalized for medical problems prior to receiving amsacrine, and six of these patients received parenteral antibiotics for documented bacterial infections. Thirty-one of 32 patients survived > 2 weeks and were thus considered evaluable for response.

All patients with ANLL or ALL had received aggressive initial therapy as previously described (12,13). Of the 21 patients with ANLL, 19 had achieved prior CR. Sixteen patients had received previous reinduction therapy following relapse. Seven of these patients had achieved CR with reinduction therapy. All 21 patients had received anthracycline chemotherapy and were considered resistant to anthracyclines. All nine patients with ALL had received reinduction chemotherapy, with five achieving CR. All patients had received anthracycline therapy. Both patients with CML-BC had received previous induction therapy with anthracycline-containing regimens.

Treatment Regimen

Patients received 120 mg/m² of amsacrine in 500 ml of 5% dextrose in water iv over 1 hour daily for 5 consecu-

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tive days. The total dose for each 5-day course was 600 mg/m^2. Twelve patients received amsacrine via central venous catheters and the remainder through peripheral venous lines. A bone marrow aspiration and a biopsy were required on the 7th day following the start of treatment and weekly thereafter until the patient was determined to have developed progressive disease or achieved a response.

Response and Toxicity

All patients who survived 2 weeks following completion of one course of therapy and had one posttreatment marrow examination were considered evaluable for response (31 of 32 patients). Patients were evaluated for CR and partial remission (PR) using the standard criteria of the Cancer and Acute Leukemia Group B (CALGB). CR was defined as a normally cellular marrow of < 5% blasts, a granulocyte count of > 2000/μl, and a platelet count of > 100,000/μl. PR was defined as a normally cellular marrow with between 5.1% and 25% blasts, a granulocyte count of > 1000/μl, and a platelet count of > 50,000/μl.

Patients were evaluated for toxicity with daily or twice weekly serum chemistries and blood cell counts. To detect possible cardiac arrhythmias during administration of amsacrine, patients had cardiac monitoring during the 5 days of drug administration and for at least 1 subsequent day. Serum electrolytes were measured daily and any abnormalities corrected prior to administration of amsacrine. The observed toxic effects were graded according to standard CALGB criteria.

RESULTS

Response

Thirty-one of the patients were considered evaluable for response. The nonevaluable patient died of refractory sepsis within 1 week of therapy. The 31 patients received a total of 40 courses. Twenty-three patients received one course, seven received two courses, and one received three courses of amsacrine therapy. No patient achieved CR, and one (with adult ALL) achieved PR lasting 3 months. A summary of the therapeutic results with amsacrine is shown in table 1. Absolute drug resistance was the most common outcome (17 of 31 patients). Five patients had relative drug resistance, with a transient but only moderate decrease in marrow blasts. The remaining nine patients developed marrow aplasia by Day 14. Five patients died without evidence of marrow recovery. Three patients developed recurrent leukemia and the final patient achieved PR.

Median survival was 10 weeks (range, 1-36) for all patients entered in the study from the beginning of amsacrine therapy. Median survival was 7.5 weeks for patients with ANLL and CML-BC and 16 weeks for patients with ALL.

TABLE 1.—Therapeutic response to amsacrine

	No. of patients			
	ANLL	ALL	CML-BC	Total (%)
Absolute drug resistance	12	5	0	17 (55)
Relative drug resistance	3	2	0	5 (16)
Aplasia				
Death	3	0	2	5 (16)
Leukemia	2	1	0	3 (10)
PR	0	1	0	1 (3)
CR	0	0	0	0 (0)

Toxicity

Amsacrine toxicity was substantial. The major toxic effects included: myelosuppression (100% of the patients), hyperbilirubinemia (41%), nausea and vomiting (41%), stomatitis (9%), and cardiac dysrhythmia (3%). The expected myelosuppression was universal, with all of the patients requiring platelet transfusions and 47% developing documented bacterial sepsis. Elevations in bilirubin and hepatic enzymes were generally mild and reversible. No patient developed symptoms from hepatic dysfunction or permanent hepatocellular damage.

The frequency of stomatitis was variable. Ninety percent of the patients had negligible stomatitis, while 3% had grade 2 stomatitis and 7% had grade 3.

One episode of supraventricular tachycardia occurred during therapy. This patient had no history of cardiac disease, and serum potassium was normal. Two other patients had prolongation of the QT interval documented by electrocardiography following administration of amsacrine. In both patients, the serum calcium and potassium were measured daily and remained normal. The prolonged QT interval persisted throughout the 5 days of treatment. No tachyarrhythmias developed in either patient.

One patient developed hypotension (80/60 mm Hg) during an infusion of amsacrine which responded to slowing the rate of infusion and volume administration. In this patient, there were no known factors other than amsacrine that may have contributed to hypotension. A second patient developed hypotension several hours following an infusion of amsacrine, but was receiving antibiotics for documented bacterial sepsis.

DISCUSSION

Previous studies have suggested that amsacrine is an active drug in patients with previously treated acute leukemia (1-11). Most studies have suggested that a response rate of 20%-30% can be anticipated when amsa-

crine is given as a single agent at doses ranging from 450 to 1000 mg/m^2/course. The durations of remission induced by amsacrine, however, have generally been brief, with most patients remaining in remission for only a few months. The response rate in patients with ALL has been generally similar to that observed in ANLL, and most of the patients studied so far have been reported to be anthracycline-resistant, suggesting that amsacrine might be a non-cross-resistant agent useful in relapsed acute leukemia (1,7,9). Despite these preliminary results suggesting that amsacrine is not cross-resistant with standard-drug regimens, the patient population most likely to respond to amsacrine has not been defined. In an effort to investigate the utility of amsacrine in heavily pretreated patients, one or more courses of amsacrine (600 mg/m^2/course) were administered to 32 relapsed patients.

The patients included in this study have probably been more heavily pretreated than those included in many earlier studies with amsacrine. After achieving CR with induction therapy, patients with both ANLL and ALL received maintenance therapy with multiple chemotherapeutic agents. The patients with ANLL received intensive sequential combination chemotherapy for an additional 14 months (12). This phase of therapy was divided into four sequences of drug combinations: doxorubicin/cytarabine; doxorubicin/azacitidine; prednisone/vincristine/6-mercaptopurine/methotrexate; and cytarabine (12). Thus, most patients with ANLL had received > 400 mg/m^2 of doxorubicin. Adults and children with ALL were induced into remission with vincristine, prednisone, L-asparaginase, and doxorubicin, followed by intensification therapy with the same drugs until a cumulative dose of doxorubicin (450 mg/m^2) had been obtained (13). The patients then received maintenance therapy with vincristine, prednisone, methotrexate, and 6-mercaptopurine for 30 months of continuous CR. Thus, the patients in this study had previously received high doses of multiple chemotherapeutic agents administered over a prolonged period of time. In addition, most patients had undergone previous attempts at reinduction therapy with cytarabine, doxorubicin, vincristine, or prednisone. The response rate of amsacrine in these patients was negligible. Only one PR of brief duration was seen in the 31 evaluable patients.

The toxic effects of amsacrine seen at the dose and schedule employed in this study were similar to those previously reported. Myelosuppression was severe but generally not prolonged. All patients developed life-threatening granulocytopenia and thrombocytopenia, and 15 of 32 patients developed documented bacterial septicemia. Hyperbilirubinemia has been reported by others and was not a clinical problem in this study. Supraventricular tachycardia was observed in only a single patient, and ECG abnormalities were noted in an additional two patients (14,15). These cardiac abnormalities were noted despite normalization of the serum potassium prior to administration of amsacrine in all patients.

Based on the above observations, we conclude that the use of amsacrine as a single agent at 600 mg/m^2/course in heavily pretreated adult and pediatric patients with relapsed leukemia is not a useful treatment, particularly in view of its toxic effects, including profound myelosuppression and uncommon but serious cardiac dysrhythmias which occur despite careful monitoring and normalization of serum electrolytes. These results are in contrast to those reported with untreated or less heavily treated patients with leukemia and emphasize the value of considering pretreatment as a prognostic variable in future studies with amsacrine (11).

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Recombinant Alpha-2 Interferon in the Treatment of Hairy Cell Leukemia[1]

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We treated 14 patients with hairy cell leukemia, 13 of whom had progressive disease, with recombinant alpha-2 interferon administered sc at 2 × 10^6 units/m^2, three times per week. Thirteen patients were evaluable for response. All evaluable patients responded within 6–8 weeks. After a minimal treatment duration of 6 months and a maximal of 12 months, three patients have achieved complete response and ten have achieved partial response. With a median treatment duration of 10 months, the responding patients' hematologic parameters are continuing to improve, and no responding patients have relapsed. This outpatient self-administered regimen is well-tolerated, with mild fever, myalgias, and headache usually resolving within 2 months. Although the optimal regimen and the mechanism of action are unknown, recombinant alpha-2 interferon may be the treatment of choice for patients whose disease progresses after splenectomy or who are not surgical candidates. [Cancer Treat Rep 69:791–793, 1985]

Hairy cell leukemia (HCL) is associated with splenomegaly, pancytopenia, and an increased incidence of fatal bacterial or fungal infections (1–6). In 60%–75% of patients, splenectomy results in hematologic improvement and increased survival, but the disease usually progresses, necessitating further therapy (7). Various treatments, including high-dose chemotherapy (8–10), low-dose chlorambucil (11), leukapheresis (12), and others (13,14), are effective in a minority of patients, and are associated with significant myelosuppression and infection. Quesada et al (15) demonstrated that daily im injections of a partially purified preparation of human leukocyte interferon induced significant responses in seven patients with HCL (15). Recently, Ratain et al (16) reported that of nine patients with HCL treated with 2 × 10^6 units/m^2 of recombinant alpha-2 interferon sc, one died after two doses and seven of eight responded. The present study was undertaken to determine the effectiveness of a uniform, low-dose, outpatient regimen of highly purified recombinant alpha-2 interferon in patients with progressive HCL.

MATERIALS AND METHODS

Fourteen consecutive patients with HCL were treated with alpha-2 interferon for at least 6 months at the University of Washington. One patient was not evaluable because of controversy as to the diagnosis. The diagnosis of HCL was based on the clinical presentation, on cell morphology, and on the presence of tartrate-resistant acid phosphatase staining hairy cells in peripheral blood (ten patients), in bone marrow biopsy specimens (13 patients), or in splenectomy specimens (12 patients). The median age was 63 years (range, 35–74), with 11 males and two females. Twelve patients had had a splenectomy and five had received prior chemotherapy or hormonal therapy. Twelve patients had progressive HCL, with falling blood cell counts and/or massive retroperitoneal lymphadenopathy.

The alpha-2 interferon (Schering Corporation) was produced by *Escherichia coli* genetically engineered to possess plasmid DNA hybridized with a human alpha-interferon gene (17). The interferon is > 95% pure and has a specific activity ≥ 1.0 × 10^8 IU/mg of protein. Alpha-2 interferon was given sc at 2 × 10^6 IU/m^2 three times per week; it was self-administered in most patients. A cbc with differential and platelet count were performed weekly. Since attempted bone marrow aspirations yielded dry taps in all patients, bone marrow biopsies were obtained before interferon therapy, after 3 months of interferon treatment, and between 9

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and 12 months of therapy. Biopsies were examined morphologically for hairy cells and for tartrate-resistant acid phosphatase activity (18).

Complete response (CR) was defined as normalization of hematocrit (≥ 36%), neutrophil count (≥ 1500/μl), and platelet count (≥ 100,000/μl); absence of transfusion requirement; and disappearance of hairy cells from the blood and bone marrow. Partial response (PR) was defined as the normalization of previously subnormal hematocrit and/or neutrophil count and/or platelet count or a > 90% reduction of hairy cells in the blood or marrow.

RESULTS

All patients treated had an objective response. Three patients achieved CR (at 10, 9, and 6 months), and ten achieved PR. The hematologic response results are summarized in table 1. After 3 months of treatment, the number of patients with neutrophil counts < 500/μl decreased from five to zero, the number with platelet counts < 100,000/μl decreased from eight to zero, and the number dependent on rbc transfusions decreased from seven to zero. Circulating hairy cells decreased dramatically within 2-4 weeks.

With a median treatment duration of 10 months, previously subnormal hematologic parameters are continuing to improve, and no responding patients have relapsed. Two patients had massive retroperitoneal adenopathy before treatment. In both patients, a 50% reduction in nodal size after 2 months of interferon therapy was documented by computerized tomographic scan.

Side effects of treatment included fever and an influenza-like syndrome of myalgia, headache, and arthralgia. These symptoms were not present before interferon therapy. Treatment-related symptoms decreased and were virtually absent after 2 months of therapy. Mild fatigue did not interfere with patients' activity. Serious infections occurred in only two patients (lobar pneumonia and perirectal abscess) during the 1st month of therapy in association with pre-existent neutropenia, and resolved with antibiotic therapy and hematologic improvement. SGOT and SGPT elevations occurred in six patients but never to more than twice the normal level. Three male patients receiving interferon developed painful bilateral gynecomastia and evidence of primary gonadal failure with elevated gonadotropins.

DISCUSSION

The earlier, encouraging report by Quesada et al (15) suggested an important role for human leukocyte interferon in therapy for HCL. Our results using highly purified, recombinant alpha-2 interferon extend that observation and demonstrate that the antitumor effect is induced by interferon per se, excluding the possibility that the antitumor effect is due to a contaminating substance. Alpha-2 interferon induced significant responses in 13 patients with HCL, most of whom had progressive disease after splenectomy. Even after 12 months of treatment, the patients' hematologic parameters are continuing to improve, and maximal clinical response may not yet have been reached. These results have been obtained with an empirically derived regimen; further clinical trials will be required to establish the optimal dose, route, and duration of interferon therapy. This trial demonstrates that alpha-2 interferon is an excellent treatment for patients with progressive disease after splenectomy, and should be the treatment of choice for patients unable to undergo splenectomy. A larger clinical trial would be required to determine whether alpha-2 interferon is *preferable* to splenectomy as first-line therapy.

The mechanism responsible for the observed antitumor effect is not known. However, the dramatic response of such slow-growing tumors to very small amounts of interferon suggests a mechanism in addition to, or separate from, a direct cytotoxic effect. Possible mechanisms include the regulation of oncogene expression (19), effects on expression of cell surface tumor-associated antigens (20), and/or enhancement of host immunologic reactivity (21,22).

TABLE 1.—Hematologic responses in 13 patients*

		Mos of therapy			
	Pretreatment $n = 13$	3 $n = 13$	6 $n = 13$	9 $n = 11$	12 $n = 4$
Hematocrit, %	30 (24-48)	35 (28-45)	42 (35-47)	42 (38-49)	44 (40-47)
Neutrophil count/μl	960 (120-4690)	1500 (760-4000)	2300 (540-5100)	2700 (800-3900)	2800 (1100-4900)
Platelet count, × 10^3/μl	95 (11-440)	227 (153-380)	280 (177-413)	286 (145-432)	326 (263-343)
Hairy cells in marrow, %	90 (11-95)	14 (< 5-90)	Not done	†	†

* Values = median (range).

†All patients had bone marrow biopsies between 9 and 12 mos of therapy and have 3% (range, 0%-65%) hairy cells in marrow.

In conclusion, the response rate of HCL to recombinant alpha-2 interferon is very encouraging. Longer follow-up is required to determine the maximal response and the duration of response. Additional trials are necessary to establish the most effective regimen, and laboratory research is needed to identify the mechanism for the antitumor effect and to determine the basis for the unique sensitivity of HCL to interferon.

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Phase I Study of Vinblastine and Verapamil Given by Concurrent Iv Infusion[1,2,3]

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Overcoming resistance to chemotherapy is an important goal in cancer treatment. In many systems, resistance to anthracyclines and vinca alkaloids correlates with a diminished intracellular content of drug. In P388 leukemia and Ehrlich ascites tumor, an active outward transport of anthracyclines and vinca alkaloids occurs. Calcium channel blockers, such as verapamil, inhibit this active outward transport and increase intracellular content of vinblastine and anthracyclines in cells resistant to vinca alkaloids and anthracyclines, respectively. We report a phase I trial of vinblastine (1.5 mg/m^2 daily as iv continuous infusion × 5 days) in 17 patients and concurrent verapamil in escalating doses. Verapamil was administered as a loading dose (0.02–0.1 mg/kg) followed by a maintenance infusion (0.036–0.18 mg/kg/hour) for 5½ days with continuous cardiac monitoring. There was no apparent augmentation of vinblastine toxicity when vinblastine and verapamil were given concurrently. ECG change was the dose-limiting toxicity. At 0.12 mg/kg/hour, five of nine patients developed first-degree heart block (mean P-R interval, 0.32 seconds; range, 0.23–0.52 seconds). Junctional rhythms were noted in two of 17 patients. Reversible nonspecific T-wave changes were seen in four of 17 patients. Blood pressure and left ventricular ejection fractions (ultrasonic) were not altered. Five of 17 patients had wbc count nadirs < 2000/mm^3, and two of 17 patients had platelet count nadirs < 100,000/mm^3. Four patients experienced neurotoxicity. A mean vinblastine concentration of 2.2 ng/ml (0.55 nM) and a mean verapamil concentration of 290 ng/ml (0.45 μM) were achieved with the concurrent 5-day infusion. The tolerable levels of verapamil obtained appear to be less than those which were reported to inhibit vinblastine efflux in vitro. Additional in vitro experiments at the tolerable doses of vinblastine and verapamil are recommended. [Cancer Treat Rep 69:795–799, 1985]

Reciprocal cross-resistance between dactinomycin, the anthracycline antibiotics, and the vinca alkaloids has been reported in several cell lines (1). This resistance has been correlated with a significantly diminished intracellular drug content in resistant cells as compared to sensitive cells (2–4). In vitro studies indicate that energy-dependent extrusion of anthracyclines and vinca alkaloids occurs in P388 leukemia and Erhlich ascites tumors and renders such cells resistant to the cytotoxic effects of these drugs (2–4). Prevention of such efflux with enhancement of intracellular drug concentration may overcome resistance and increase sensitivity of human tumors to these agents.

Vinblastine has shown significant activity in malignant lymphoma, breast cancer, head and neck carcinoma, testicular carcinoma, and malignant brain tumors (5,6). Since vinca alkaloids appear to be M-phase-specific (1), the cytotoxicity should be enhanced by continuous exposure of sensitive cells to the drug. This has been shown in vitro (7). A 5-day continuous infusion of vinblastine

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(1.4-2.0 mg/m^2/day) is well tolerated by most patients (8).

Verapamil is an extensively tested, potent inhibitor of transmembrane calcium flux, which influences cardiac electrophysiology, muscle excitation-contraction, and energy utilization and storage (9,10). It is customarily prescribed as an antiarrhythmic and as a coronary and peripheral arterial vasodilator.

In vivo experiments have shown increased survival time of mice treated with vinblastine plus verapamil when compared to vinblastine alone (7). These experiments have also demonstrated an increase in intracellular vinblastine in cells treated with verapamil.

Accordingly, the Wisconsin Clinical Cancer Center completed a phase I trial to determine the maximum tolerated dose of iv verapamil when administered as a 5-day continuous infusion with iv vinblastine. Plasma concentrations of verapamil and vinblastine were assessed.

MATERIALS AND METHODS

Adults with histologic evidence of advanced malignancy who were not candidates for known effective regimens or protocol treatments and had recovered from toxic effects of prior treatment were eligible for this study. Each patient had an Eastern Cooperative Oncology Group (ECOG) performance status of 3 or better and a life expectancy of at least 12 weeks. A minimum of 4 weeks had elapsed since prior treatment with chemotherapy, immunotherapy, and/or radiotherapy. Adequate bone marrow function (wbc count $\geq$ 4000/mm^3 and platelet count $\geq$ 100,000/mm^3), adequate liver function (bilirubin $\leq$ 2.0 mg/100 ml and SGOT less than twice the normal), and adequate renal function (creatinine $<$ 2.0 mg/100 ml, BUN $\leq$ 30 mg/100 ml, and normal urinalysis) were necessary prior to study entry. Pretreatment cardiac evaluation required a P-R interval $\leq$ 0.2 second, a heart rate of 60-100 beats/minute, and a normal resting left ventricular ejection fraction (ultrasonic). Informed consent was obtained from all patients according to institutional policy. Responses and toxicity were defined by standard ECOG criteria (11): Prior therapy with vinblastine did not disqualify patients from this study.

Pretreatment evaluation included a history and physical examination with documentation of all measurable disease. Laboratory studies included a wbc count with differential, hematocrit, hemoglobin, platelet count, prothrombin time, chemistry panel (BUN, creatinine, calcium, phosphorus, uric acid, alkaline phosphatase, SGOT, LDH, and total bilirubin), urinalysis, and appropriate radiologic examinations. A baseline ECG and measurement of resting ultrasonic left ventricular ejection fraction were obtained. Weekly wbc counts, differentials, hematocrits, hemoglobins, platelet counts, prothrombin times, urinalyses, and chemistry panels were required. ECGs were performed daily while patients received verapamil, and a resting left ventricular ejection fraction was measured just prior to discontinuing the verapamil in each course. Repeat physical examination, including a neurologic examination and tumor measurements, was obtained every 3-4 weeks.

Vinblastine was administered continuously in a 5% dextrose-water solution at a dose of 1.5 mg/m^2/24 hours for 120 hours (5 days). Verapamil was given iv by bolus over 1 minute followed by a continuous infusion in a 5% dextrose-water solution for 132 hours delivered by an IVAC pump beginning at the same time as vinblastine. The initial dosage was 0.02 mg/kg iv bolus over 1 minute and 0.036 mg/kg/hour as a continuous infusion. Escalations were planned to dosages of 0.04, 0.07, 0.10, 0.14, and 0.18 mg/kg as the initial iv bolus, and 0.06, 0.12, 0.18, 0.24, and 0.30 mg/kg/hour as the continuous infusion according to a modified Fibonacci scheme. The bolus injection of verapamil was given to more rapidly achieve steady-state drug concentrations. Since the half-life of verapamil is shorter than that of vinblastine, the verapamil infusion was maintained for 12 hours after discontinuing the vinblastine infusion (132 vs 120 hours, respectively).

Cycles of therapy were repeated every 21 to 28 days. Dose escalations were not permitted within the same patient.

Limited pharmacokinetic evaluation was performed in selected patients at the maximum tolerated dose level. Peripheral venous blood samples were drawn twice daily during the infusion period. Heparinized plasma was prepared and frozen for analysis of intact verapamil and vinblastine.

Plasma samples were analyzed for vinblastine by a previously published radioimmunoassay technique (12). The antivinca antiserum (Lot No. 24-245-2-R) was graciously provided by Dr. Mary Ruth of Eli Lilly Research Laboratories, Indianapolis, IN. Tritiated vinblastine sulfate was purchased from Amersham Corporation, Arlington Heights, IL, and had a specific activity of 10.4 Ci/mM. Verapamil concentrations in the plasma of treated patients were determined by a modification of McAllister's gas-liquid chromatography method with a nitrogen-phosphorus selective detector (13).

RESULTS

Seventeen eligible patients were entered in this phase I trial. A total of 33 courses of vinblastine and verapamil was administered. There were sufficient data from 31 of these courses to permit full evaluation for toxicity; the verapamil infusion was aborted during the two remaining courses. One patient received 2 days of therapy (verapamil dose of 0.18 mg/kg/hour). Treatment was terminated because of a junctional rhythm with frequent premature ventricular beats. A second patient completed 1

day of his second cycle (verapamil dose of 0.036 mg/kg/hour). He developed sudden respiratory distress resulting in discontinuation of the infusion, and died 16 days later secondary to *Pseudomonas* pneumonia. This patient was also diagnosed as having hypothyroidism, and began replacement therapy 1 week prior to death.

Eleven patients had received prior chemotherapy and radiotherapy, five were previously treated with chemotherapy alone, and one had no prior treatment. An ECOG performance status of 0 or 1 was noted in 11 patients. Six patients presented with an ECOG performance status of 2 or 3. Patients had advanced solid tumors of the types shown in table 1. There were no objective complete or partial responses recorded during the infusion trial.

Mild malaise was experienced by five patients; moderate to severe malaise was seen in six patients at all dose levels. Five patients noted mild to moderate nausea and vomiting. Seventy-six percent of patients (13 of 17) had received a prior, potentially neurotoxic drug including vincristine, vinblastine, cisplatin, hexamethylmelamine, acivicin, carboplatin, and misonidazole; however, only four patients developed neurologic symptoms during the vinblastine/verapamil infusion (table 2). One patient, who had had previous treatment with acivicin, misonidazole, and carboplatin, and a second patient with previous dacarbazine therapy required 50% and 25% vinblastine dose reductions, respectively, during the second treatment course because of paresthesias. Generalized myalgias were experienced by a third patient who had received prior vinblastine and misonidazole following the second infusion course. Increased lower extremity weakness was noted in a fourth patient who had had previous vincristine therapy, resulting in a 50% vinblastine dose reduction during the second treatment course.

Hematologic toxicity was consistent with previous reports utilizing vinblastine as a single agent (8) (table 2). The median wbc count nadir range was 1700–3900/mm^3; the median platelet count nadir range was 159,000–250,000/mm^3. One heavily pretreated patient experienced a wbc count nadir of 900/mm^3 and a platelet count nadir of 59,000/mm^3 (verapamil dose of 0.12 mg/kg/hour). Subsequent vinblastine doses were reduced by 50% without further myelosuppression. Two other patients required a 25% vinblastine dose reduction because of leukopenia (wbc count nadirs of 1400 and 1900/mm^3, respectively), one of whom also had thrombocytopenia (platelet count nadir of 87,000/mm^3). Cumulative hematologic toxicity was not noted.

ECG changes were dose-limiting in this trial (table 3). Five of nine patients developed first-degree heart block (maximum P-R mean, 0.32 seconds; range, 0.23–0.52 seconds) at a verapamil infusion rate of 0.12 mg/kg/hour. From this group of five patients, one was given an initial verapamil infusion rate of 0.18 mg/kg/hour; however, when her P-R interval increased to 0.4 seconds, the verapamil was decreased to 0.12 mg/kg/hour. The P-R interval at the decreased infusion rate was 0.28 seconds. A second patient from this group exhibited first- and second-degree heart block with a wandering atrial pacemaker, an occasional junctional rhythm, and premature ventricular beats during each of four courses. Atrial bigeminy and first-degree atrioventricular block developed in a third patient whose serum potassium was 2.9 mmols/L. Verapamil was discontinued on Day 2 of infusion Course 1. The infusion was not resumed, since an episode of ventricular tachycardia was recorded on Day 3. A fourth patient, who later died from *Pseudomonas* pneumonia, presented with a pretreatment ECG showing right bundle branch block and a probable old anterolateral myocardial infarction; these abnormalities were unchanged during the infusion course. ECG from the fifth patient demonstrated pretreatment right bundle branch block, and first-degree atrioventricular block with T-wave changes during the infusion.

The verapamil infusion (0.18 mg/kg/hour) was discontinued during Course 1, Day 3 in one patient because of a junctional rhythm with escape beats and frequent premature ventricular beats. His predrug ECG showed a possible old inferior myocardial infarction. Sinus tachycardia was recorded in eight patients at all dose levels of verapamil, and four patients had nonspecific T-wave changes.

Blood pressures were recorded every 6 hours and did not differ significantly for any patient during any infusion course. Pre- and post-infusion cross-sectional echocardiograms were performed in 15 patients to quantitate

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	17
Sex	
Males	6
Females	11
Mean age in yrs: 58	
Performance status (ECOG)	
0–1	11
2–3	6
Prior therapy	
Chemotherapy	5
Radiotherapy	0
Both	11
Neither	1
Neurotoxic chemotherapy	13
Tumor type	
Breast	9
Pancreas	2
Lung	1
Colon	1
Uterine	1
Renal	1
Sarcoma	1
Carcinoid	1

TABLE 2.—Noncardiac toxic effects

Total dose of verapamil (mg/kg)	No. of patients entered	Toxic effects*				
		Malaise	Nausea/ vomiting	Neurologic	Leukopenia†	Thrombocytopenia‡
5	3	2	2	1	1	1
8	3	2	1	0	0	0
16	9	6	2	2	3	1
24	2	1	0	1	1	0

*Values = No. of patients.
†Wbc count < 2000/mm³.
‡Platelet count < 100,000/mm³.

left ventricular ejection fractions. All 17 patients obtained at least one echocardiogram; however, one patient did not receive a preinfusion and another did not receive a postinfusion ejection fraction evaluation. Three patients also required one radionuclide ventriculography because of a technically unsatisfactory echocardiogram. In all cases, pre- and post-infusion left ventricular function was normal and did not significantly vary in value. Incidental findings included asymptomatic mitral valve prolapse (two patients), a calcified mitral annulus with thickened aortic leaflets and a hypertrophied right ventricle (one), and a thickened aortic root and leaflets (one).

A mean plasma vinblastine concentration of 2.2 ± 2.0 ng/ml (0.55 nM) was achieved during the 5-day infusion (1.5 mg/m²/day). This level is comparable to that obtained by Young et al (14), 2.2 ng/ml ± 0.96 ng/ml, using the same dose and schedule.

Verapamil concentrations of 290 ± 100 ng/ml (0.45 μM) were achieved during the 132-hour infusion (0.12 mg/kg/hour). During a 12-hour infusion at 0.125 mg/minute, Reiter et al (15) noted a mean verapamil concentration of 122 ± 21 ng/ml.

DISCUSSION

Tsuruo and colleagues (7) have attempted to overcome vinca alkaloid resistance in vinca-resistant P388 murine leukemia (P388/VNC) using verapamil. Mice bearing P388/VNC and treated with vinblastine plus verapamil survived 30% longer compared to those treated with vinblastine alone. In vitro studies demonstrated a tenfold reduction in the concentration of vinblastine necessary to inhibit growth of 50% of P388/VNC colonies (IC_{50}) in verapamil-treated cells compared to controls. There were no apparent changes in membrane permeability, tubulin binding, or drug influx. A significant increase in the intracellular content of vincristine and vinblastine in P388/VNC cells treated with verapamil was noted. Increased intracellular vinca concentrations resulted in cytotoxicity that was similar to that seen in sensitive P388 cells and appeared to be mediated by inhibition of drug efflux by verapamil (7).

The ability of verapamil to overcome drug resistance in patients may be limited by toxicity. Prolongation of atrioventricular nodal conduction lasts up to 6 hours after an iv injection of verapamil (16). Iv injections of 0.10 and 0.15 mg/kg may lengthen the measured atrio-HIS bundle conduction time 115% and 127%, respectively (17,18). There has been no effect of verapamil on atrial or ventricular conduction. The intrinsic negative inotropic effects of verapamil, which include transient increases in heart rate, decreases in mean arterial pressure, decreases in peripheral vascular resistance, and increases in cardiac index (19–25), are clinically insignificant.

Our clinical phase I study suggests that verapamil does not enhance vinblastine toxicity. Drug-related gastrointestinal discomfort, paresthesias, leukopenia, and thrombocytopenia were consistent with toxicity reported in other patients receiving a 5-day continuous iv vinblastine infusion at 1.5–1.8 mg/m²/day (8).

Verapamil, at a maximum dosage of 0.18 mg/kg/hour, caused no alteration in blood pressure or left ventricular ejection fraction. Sinus tachycardia was noted in eight of 17 patients. Previous investigators suggested that the

TABLE 3.—ECG changes

Total dose of verapamil (mg/kg)	No. of patients entered	ECG changes*				
		PVCs†	Junctional rhythm	P-R interval > 0.2 secs (range, 0.23–0.52 secs)	Sinus tachycardia	T-wave changes
5	3	1	0	0	1	3
8	3	0	0	0	1	0
16	9	2	1	4	5	1
24	2	1	1	1	1	0

*Values = No. of patients.
†PVC = premature ventricular contraction.

lowest dose of verapamil to exhibit any hemodynamic effect was a 0.1-mg/kg iv bolus followed by a maintenance drip of 0.3 mg/kg/hour (24).

Sustained P-R interval prolongation is usual during continuous-infusion verapamil when plasma verapamil concentrations are maintained at steady-state concentrations of 77-174 ng/ml (mean, 122 ng/ml) (14). Five of nine patients in this study developed a sustained P-R interval > 0.2 seconds during the continuous verapamil infusion rate of 0.12 mg/kg/hour (with prior bolus of 0.07 mg/kg); however, all P-R intervals returned to a normal range upon discontinuation of the infusion.

Tsuruo et al (7) employed noncytotoxic concentrations of verapamil (2.2-6.6 μM) to enhance the cytotoxicity of vinca alkaloids in P388 and P388/VNC cell lines. At verapamil concentrations of 2.2 and 6.6 μM, the vinblastine concentrations required to inhibit 50% of the P388 colonies (IC_{50}) were 1.6 and 0.45 nM, respectively. The vinblastine IC_{50} in P388/VNC cells was 1.7 and 0.34 nM at verapamil concentrations of 2.2 and 6.6 μM, respectively. Our clinical trial suggests that at the maximum tolerated dose of verapamil, plasma concentrations of 0.45 μM verapamil and 0.55 nM vinblastine are achieved during the infusion. This verapamil concentration is, at the minimum, five times lower than that reported by Tsuruo to enhance vinblastine cytotoxicity in vitro.

In conclusion, there was no additive myelotoxicity seen with the vinblastine/verapamil combination. The dose-limiting toxicity was first-degree heart block. Additional clinical and in vitro data are needed to determine the drug concentrations which are sufficient to increase intracellular vinblastine uptake in resistant cells.

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Phase I Clinical Trial of Mitozolomide[1,2,3]

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Mitozolomide (NSC-353451; CCRG 81010; M and B 39565) is a novel potential anticancer agent that was selected for phase I study on the basis of broad spectrum activity in mouse tumors. Initially, mitozolomide was given iv as a short infusion to 37 patients in doses ranging from 8 to 153 mg/m^2. Nausea and vomiting was dose-related but was not severe. The dose-limiting toxic effect was thrombocytopenia at doses > 115 mg/m^2, and recovery from the thrombocytopenia was delayed up to 8 weeks. Partial responses were seen in two patients with adenocarcinoma of the ovary. The pharmacokinetics of mitozolomide showed that the half-life of the intact drug in the plasma was between 1 and 1.3 hours. The area under the curve was proportional to the dose administered. Mitozolomide is well-absorbed; therefore, future studies are recommended using a single-dose schedule orally or iv. In the phase I study reported here, a dose of 115 mg/m^2 appeared to be safe, but additional studies have shown that when given orally to an older population, most patients experienced thrombocytopenia < 50,000 cells/mm^3. The recommended dose using current data is 90 mg/m^2 iv or orally. [Cancer Treat Rep 69:801–805, 1985]

Mitozolomide [8-carbamoyl-3-(2-chloroethyl)imidazo[5,1-D]-1,2,3,5-tetrazin-4(3*H*)-1] (fig 1) was first synthesized in 1980 (1) as part of a continuing program concerned with investigating the chemical and biological properties of small molecules bearing NNN linkages (2,3). The drug has been tested against a wide range of murine tumors; it produced 60-day cures in animals inoculated with L1210 and P388 leukemias, and displayed pronounced activity (++ on the National Cancer Institute scale) against the B16 melanoma, colon 38 tumor, Lewis lung carcinoma, and the LX-1 lung tumor xenograph (1).

Studies concerned with evaluation of the chemical properties of mitozolomide (1) have indicated that, in aqueous solutions, the molecule is exquisitely sensitive to changes in pH, exhibiting prolonged stability in acidic environments, whereas in alkaline solution, the molecule is rapidly degraded (1,4). Investigation into the fate of the degraded molecule identified the acyclic monochloroethyltriazene (MCTIC), 5-[3-(2-chloroethyl)triazene-1-yl]-imidazole-4-carboxamide, as a significant product (1). The chloroethyltriazene TIC-mustard, 5-[3,3-bis(2-chloroethyl)triazene-1-yl]-imidazole-4-carboxamide, has been shown to have promising experimental antitumor activity (5), and its potential metabolic decomposition product, MCTIC, has been implicated in this activity (6). However, the inherent chemical instability of these acyclic chloroethyltriazenes has deterred their widespread clinical evaluation.

The possibility that mitozolomide may be exerting its antitumor effect by acting as a stable prodrug of MCTIC has been investigated in a number of experimental systems. Both mitozolomide and MCTIC have been shown to produce similar effects on cell cycle progression, as evidenced by flow cytometry, and both the parent drug and its postulated active species exhibit equivalent in vitro cytotoxicity and DNA-alkylating ability when examined by alkaline elution (7–9).

The unique chemical properties of mitozolomide, in ad-

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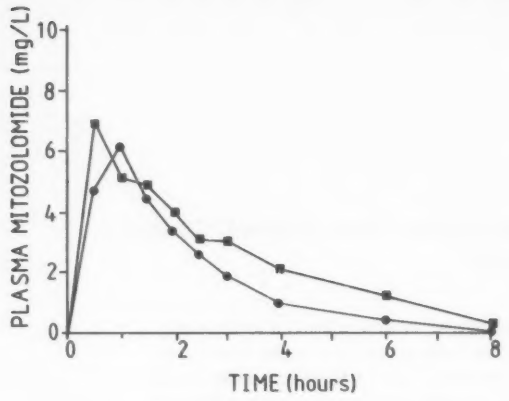

FIGURE 1.—Mitozolomide plasma concentration vs time profiles for Patient 31. ● = iv dose; ■ = oral dose.

dition to the pronounced activity against murine tumor models, stimulated the activation of a phase I clinical trial.

MATERIALS AND METHODS

Mitozolomide for clinical use was supplied to the Department of Pharmaceutical Sciences, University of Aston, Birmingham, by May and Baker, Ltd, Dagenham, Essex. The iv preparation was formulated as either a 5% or 10% solution in DMSO. Ampoules were stored at -20°C. Mitozolomide was administered as a 1-hour infusion in 500 ml of normal saline. Handling precautions of the iv preparation included wearing two pairs of latex gloves, preparing the drug in a safety cabinet, and using polypropylene syringes and polyfusor plastic to avoid the solvent action of DMSO. For future studies, the oral formulation of mitozolomide will be provided as hard gelatin capsules.

All patients had advanced cancer refractory to known methods of therapy, and a life expectancy of at least 2 months. All patients gave written informed consent prior to entry in the study. The minimum hematological requirements were a wbc count > 4000/mm^3 and a platelet count > 100,000/mm^3. Patients were required to have normal liver function tests, urea and electrolytes, serum creatinine, uric acid, glucose, blood clotting screen, urinalysis, and ECG. Following administration of mitozolomide, a full blood cell count was done twice weekly; at weekly intervals, the patients were seen for clinical examination and rechecking of the coagulation screen, liver function tests, urea and electrolytes, serum creatinine, uric acid, and glucose. Partial response was defined according to the World Health Organization (WHO) classification.

In the absence of toxicity, a second dose was given on Day 22 in the first part of the study. Dose escalation was permitted, but these patients did not form part of the initial entries at the new dose level. At higher doses, a further dose of mitozolomide was delayed to between 6 and 8 weeks depending on recovery from thrombocytopenia. The starting dose was based on the murine toxicology. The initial dose was 8 mg/m^2, and increments were increased as a modified Fibonacci search to a maximum of 153 mg/m^2.

The pharmacokinetics were studied in selected patients at each dose level, and blood (5–10 ml) was drawn at the following times from the beginning of the 1-hour infusion: 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0, and 24 hours. All samples were stored at -20°C until analysis. Mitozolomide concentrations in plasma were measured by high-pressure liquid chromatography (4,10).

Estimation of Pharmacokinetic Parameters

The elimination rate constants (k_{el}) were determined by linear regression analysis of plots of ln (plasma concentration) versus time (slope = $-k_{el}$). k_{el} was used to extrapolate the plasma concentration at the end of the infusion (Cp_{ex}). The total area under the plasma concentration versus time curve ($AUC_{0-\infty}$) was determined by the addition of the postinfusion

$$\text{AUC } \left(\frac{Cp_{ex}}{k_{el}}\right)$$

to the AUC during infusion, which was obtained from the expression

$$Cp^{\infty}\left[T - \frac{(1-e^{-k_{el}T})}{(k_{el})}\right]$$

where T was the infusion time and Cp^{∞} was obtained from the expression

$$\frac{Cp_{ex}}{(1 - e^{-k_{el}T})}.$$

The plasma half-lives were obtained from

$$\frac{0.693}{k_{el}}$$

and the plasma clearance was obtained from

$$\frac{\text{Dose}}{\text{AUC}_{0-\infty}}.$$

The volume of distribution was obtained from

$$\frac{\text{plasma clearance}}{k_{el}}.$$

For oral bioavailability studies, all of the AUCs were estimated by the trapezoidal rule, with an extra time point obtained 0.5 hour from the beginning of the infusion (or oral administration of the capsules).

RESULTS

A total of 37 patients (15 males and 22 females) were entered in the study. The median age was 54 years (range, 17–75). The following tumor types were included: adenocarcinoma of the ovary (ten patients); renal cell carcinoma (four); colorectal (four); unknown primary (three); lung (three); osteosarcoma (two); non-Hodgkin's lymphoma (two); thyroid (two); and squamous cell carcinoma of the head and neck, carcinoma of the stomach, testicular carcinoma, squamous cell carcinoma of the cervix, bladder carcinoma, melanoma, and carcinoid tumor (one each). Five patients had received prior radiotherapy, 17 had received prior chemotherapy, 14 had received both prior radiotherapy and chemotherapy, and one had received no prior treatment.

Nausea and vomiting was defined as mild (nausea and one to two episodes of vomiting), moderate (nausea and vomiting lasting up to 4 hours and controlled by antiemetics), or severe (vomiting lasting 4 hours, requiring parenteral support). At doses up to 82 mg/m^2, nausea and vomiting was either absent or mild; at higher doses, 17 of 28 (61%) patients experienced moderate vomiting, but in no patient was the vomiting severe.

The dose-limiting toxic effect of mitozolomide was myelosuppression, with thrombocytopenia being more pronounced than leukopenia (table 1). Minimal myelosuppression was encountered at doses up to 115 mg/m^2, at which three of eight patients experienced thrombocytopenia of 50,000–74,000 cells/mm^3 (two of these patients had received mitozolomide at a lower dose). At 115 mg/m^2, one patient experienced leukopenia of 1000–1900 cells/mm^3. At the next dose level of 153 mg/m^2, thrombocytopenia was profound and prolonged (seven of eight patients had recovered a platelet count of > 100,000/mm^3 by 8 weeks). Subsequent dosage reductions to 140 and 125 mg/m^2 again confirmed that the dose-limiting toxic effect was thrombocytopenia, which again showed a delayed recovery of 6–8 weeks. One patient who had a total of seven doses of mitozolomide experienced some exacerbation of previous cisplatin-induced peripheral neuropathy. No other side effects were encountered in any of the patients.

Two patients with adenocarcinoma of the ovary who had received prior cisplatin therapy had partial responses (WHO) in measurable intra-abdominal lesions lasting 3 and 6+ months. One patient with non-Hodgkin's lymphoma experienced a transient reduction in lymphadenopathy.

The essential pharmacokinetic parameters of mitozolomide obtained from this study are summarized in table 2. The plasma half-life was in agreement with that predicted from earlier animal studies (10), with a volume of distribution (36.4 L) approximating that of total body water. In most of the 12- and 24-hour samples, the concentration of mitozolomide had fallen below the sensitivity limit of the assay (0.01 mg/L). There was no evidence for dose dependency in the pharmacokinetics of mitozolomide, with plots of dose (mg/m^2) against maximum plasma concentration and $AUC_{0-\infty}$ giving correlation coefficients of 0.985 and 0.993, respectively.

TABLE 1.—Myelosuppression with mitozolomide at each dose level

Dose (mg/m^2)	No. of patients/ No. of courses	Median nadir count (range)*		
		Wbc	PMN†	Platelet
8	6/6	5.25 (4.8–10.6)	5.1 (3.0–7.9)	225 (20–428)‡
16.5	8/9	5.5 (4.3–10.6)	5.3 (2.5–13.0)	302 (169–484)
33	8/11	7.6 (3.4–10.6)	5.0 (2.5–8.7)	289 (139–523)
54.4	5/7	5.7 (2.5–8.0)	4.5 (3.8–6.2)	170 (60–467)
82	4/4	3.5 (2.6–4.3)	2.8 (2.2–3.3)	175 (135–202)
115	8/10	4.3 (1.5–8.2)	2.9 (0.9–5.7)	116 (52–208)
125	5/5	2.5 (1.2–6.8)	1.8 (0.25–5.8)	21 (7–75)
140	5/5	3.7 (1.1–5.2)	2.8 (1.3–7.9)	42 (21–129)
153	10/10	1.7 (0.5–4.2)	0.9 (0.04–2.1)	22 (12–141)

* Values = × 10^9 cells/L.
†Polymorphonuclear neutrophils.
‡Thrombocytopenia occurred in 1 patient from infection from bowel perforation.

TABLE 2.—Essential pharmacokinetic parameters of mitozolomide

No. of patients/dose	Dose (mg/m^2)	C_{max} actual (mg/L)*	AUC 0-∞ (mg·hr/L)	Plasma half-life (hrs)†
6	8.0	0.425	0.855	1.203
7	16.5	0.830	1.411	1.116
5	33.0	1.175	2.594	1.300
5	54.4	2.054	4.864	1.064
4	82.0	3.066	6.298	1.193
6	115.0	6.103	8.834	1.140
6	153.0	7.941	13.686	1.108

* Plasma concentration of mitozolomide at end of infusion.
†Average = 1.161; SD = 0.078.

At lower doses, there was a good fit to an open one-compartment model, with no significant differences between the actual concentration, measured at the end of the infusion, and that predicted using the elimination rate constant. However, at the two highest doses, 115 and 153 mg/m^2, the predicted concentrations at the end of the infusion were 4.164 and 6.511 mg/L, respectively. The actual concentrations (table 2) at the end of the infusion indicate that a small distribution phase may be present. However, the lack of sufficient data points immediately following the infusion precluded the accurate fitting of the data to a two-compartment model.

The oral bioavailability of mitozolomide was investigated in seven patients who received identical oral and iv doses. Three dose levels were studied (table 3), with no evidence of dose-dependent pharmacokinetics. The bioavailability data are summarized in table 3, with the plasma concentration versus time profiles for Patient 31 given in figure 1. In this case, the interval between the initial oral dose and the iv dose was 7 weeks, and although there is not a large difference in the profiles obtained, the slightly longer elimination half-life with the oral preparation is responsible for the bioavailability being 137%. If the data from Patient 31 were omitted from table 3, then the mean bioavailability falls to 95%, with a coefficient of variation of 12%.

DISCUSSION

Mitozolomide was introduced into clinical trials because of broad spectrum activity in murine tumors (1). The dose-limiting toxic effect of mitozolomide is myelosuppression, with thrombocytopenia more pronounced than leukopenia. There is delayed recovery from myelosuppression lasting up to 8 weeks at doses > 115 mg/m^2. In general, mitozolomide is a well-tolerated potential anticancer agent whose only other side effect encountered so far is dose-related but not severe nausea and vomiting. The recommended dose for further studies is 90 mg/m^2 iv at intervals of 6-8 weeks, depending on evidence of myelosuppression. Mitozolomide is well-absorbed in animals, and our data confirm this in the six patients studied so far. Current studies are evaluating the oral route of administration, and mitozolomide is well-tolerated in terms of symptoms. To try to avoid the profound thrombocytopenia that occurs with single-dose administration, six patients have been treated with 50 mg of oral mitozolomide weekly. Thrombocytopenia has developed in all of these patients after five to six doses of mitozolomide (ie, at approximately the same total dose that thrombocytopenia was induced with the single iv administration). Once studies with the ^{14}C-labeled compound are available, the distribution of metabolites may suggest alternative scheduling of this compound to try to minimize its dose-limiting toxicity.

Clinical activity has been seen in this phase I study in two of ten patients with adenocarcinoma of the ovary.

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TABLE 3.—Mitozolomide oral bioavailability

Patient No.	Dose (mg)	AUC (iv) (mg·hr/L)*	AUC (oral) (mg·hr/L)	Bioavailability (oral/iv) (%)†
31	250	14.486	19.797	136.666
36	200	9.691	9.413	97.129
38	50	2.003	2.032	101.463
39	50	2.491	1.889	75.848
40	50	2.735	2.988	109.250
41	50	2.570	2.274	88.489
42	50	2.305	2.262	98.152

* All AUC estimated trapezoidally from 0 to 8 hrs.
†Mean = 101.000; SD = 18.941; and coefficient of variation = 18.754.

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Sequentially Administered 5-Azacitidine and Amsacrine in Refractory Adult Acute Leukemia: A Phase I–II Trial of the Southeastern Cancer Study Group[1,2,3]

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The Southeastern Cancer Study Group conducted a phase I–II trial of sequentially administered 5-azacitidine and amsacrine in patients with refractory adult acute leukemia from September 1980 to March 1983. The 5-azacitidine was administered by continuous iv infusion on Days 1–4 at doses ranging from 112 to 200 mg/m²/day, while amsacrine was given at doses ranging from 75 to 150 mg/m²/day on Days 5–8. The doses of 5-azacitidine and amsacrine were alternately escalated through six dose levels during the phase I portion of the trial. Of 128 patients entered, 102 (80%) were evaluable for response. Remission was achieved in 13 of 80 evaluable patients with acute myeloid leukemia, in one of 12 evaluable patients with acute lymphoid leukemia, and in none of 11 patients with blastic transformation of chronic granulocytic leukemia. Three remissions occurred in patients with acute myeloid leukemia who were refractory to initial induction chemotherapy with cytarabine and anthracycline combination chemotherapy. Remissions were relatively durable, lasting a median of 28 weeks in the 13 patients with refractory acute myeloid leukemia (range, 14–54 weeks). Toxic effects included universal severe myelosuppression, hyperbilirubinemia at a frequency and severity similar to those seen with amsacrine used as a single agent, moderately severe stomatitis and diarrhea, three incidents of amsacrine-related cardiac dysrhythmia, and a single case of probable drug-related cardiomyopathy. This combination has activity in the treatment of myeloid leukemia, which is primarily resistant to cytarabine and anthracyclines, and could have a role in primary management. [Cancer Treat Rep 69:807–811, 1985]

Both amsacrine and 5-azacitidine have shown activity in phase II trials in the treatment of refractory adult acute leukemias (1–8). Although our previous experience with 5-azacitidine plus beta deoxythioguanosine (9) was not clearly better than with 5-azacitidine alone (6), the combination of this agent with a DNA binder was of interest. Hence, in September 1980, the Southeastern Cancer Study Group (SEG) initiated a phase I–II clinical trial

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[3]5-Azacitidine and amsacrine were provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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of sequentially administered 5-azacitidine and amsacrine in adults with refractory leukemia. The results form the basis of this report.

MATERIALS AND METHODS

Patients

Criteria for entry in the protocol included the diagnosis of acute myeloid leukemia (AML) (French, American, British classification, M1-M6), acute lymphoblastic leukemia (ALL), or blastic transformation of chronic granulocytic leukemia (BT-CGL) resistant to established therapy. Also required were the absence of residual toxic effects to prior chemotherapy, Karnofsky performance status > 40%, and bilirubin < 4 mg/dl.

Treatment Regimen

During the phase I portion of the study, the doses of 5-azacitidine and amsacrine were alternately escalated through six dose levels as indicated in table 1. 5-Azacitidine was dissolved in 200-250 ml of Ringer's lactate administered by continuous iv infusion over 8 hours repeatedly, for a total of 96 hours (Days 1-4). Immediately following, amsacrine dissolved in 250-300 ml of 5% dextrose in water was infused over a 2-3-hour period and repeated daily for a total of four doses (Days 5-8). On the days the amsacrine was given, serum potassium levels were determined and the drug was withheld if the potassium was < 3.5 mEq/L until the hypokalemia could be corrected. If the serum bilirubin was between 2 and 4 mg/dl, the amsacrine dose was reduced by 25%.

On Day 14, a bone marrow aspirate and biopsy were required and if the marrow was > 50% cellular and was comprised of > 50% immature cells, a repeat cycle was initiated. If the marrow was < 50% cellular and the immature cells comprised < 30%, the treatment was delayed and the marrow repeated in 1 week to determine if there was improvement in normal hematopoiesis or progressive leukemic involvement of the marrow. Although there was dose escalation from one level to the next during the phase I portion of the study, there was no dose escalation for individual patients. Failure with this chemotherapeutic regimen was indicated by lack of a complete remission (CR) following at least one episode of bone marrow aplasia (< 10% cellularity documented by biopsy) or 8 weeks of therapy.

Patients who achieved CR were given maintenance therapy with 5-azacitidine [50 mg/m^2 by continuous iv infusion over 8 hours repeated for a total of 48 hours (Days 1-2)] followed by amsacrine [100 mg/m^2 iv infusion over 2-3 hours for single dose (Day 3)]. Maintenance therapy was repeated every 4 weeks or when hematologic recovery occurred.

TABLE 1.—Dose escalation schedule of sequentially administered 5-azacitidine and amsacrine*

Dose level	5-Azacitidine (Days 1-4)*	Amsacrine (Days 5-8)*
1	112	75
2	112	100
3	150	100
4	150	125
5	200	125
6	200	150

* Values = mg/m^2/day.

Evaluation of Response and Toxicity

Patients who failed to receive a complete course of therapy and had no posttreatment marrow examinations or serum chemistries were considered inevaluable for both toxicity and response. Patients who received a complete course of therapy and had one posttreatment marrow examination were considered evaluable for response. CR and partial remission (PR) were determined using standard criteria. CR was defined as a normally cellular marrow with < 5% blasts and < 10% total blasts and promyelocytes, granulocyte count > 2000/μl, and platelet count > 100,000/μl. PR was defined as a normally cellular marrow with 5.1%-25% blasts, granulocyte count > 1000/μl, and platelet count > 50,000/μl.

Patients who had follow-up serum chemistries and blood cell counts were evaluable for toxicity. Possible renal and hepatic toxic effects were monitored by a minimum of weekly blood chemistries and the observed toxic effects were graded according to standard SEG criteria.

RESULTS

Therapeutic Outcome

Between September 1980 and June 1982, 65 patients were entered in the phase I portion of this protocol as doses of chemotherapy were escalated through six dose levels. Sixty-three patients were subsequently entered in the phase II portion at the highest dose level until the protocol was closed in March 1983. Patients ranged in age from 18 to 81 years, with a median of 45 years. One hundred and one patients had some form of AML, 15 had ALL, and 12 had BT-CGL. There were 70 males and 58 females entered.

Of the 101 patients with AML, 28 were primarily refractory, having failed initial induction attempts with an anthracycline-cytarabine regimen. Thirty-four patients were in their first relapse, with the remainder in their second to fifth relapse.

One hundred and two of the 128 patients entered were evaluable for response (80%). Of the evaluable patients, 13 of 80 with AML achieved CR (16%), while five achieved PR (6%). Three of these CRs occurred in 28 evaluable patients who failed initial induction chemo-

TABLE 2.—Therapeutic response to 5-azacitidine and amsacrine

Diagnosis	No. of patients		
	Entered	Evaluable	CR (%*)
AML	101	80	13 (16)
ALL	15	12	1 (8)
BT-CGL	12	11	0
Total	128	102	14 (14)

* % of patients evaluable for response.

therapy with anthracycline-cytarabine regimens. One of the 12 patients with ALL achieved CR (8%) and one achieved PR. There were no favorable responses in the 11 evaluable patients who had BT-CGL (table 2).

In table 3, the clinical outcomes of the evaluable patients entered at each of the six dose levels are presented. The clinical outcomes are categorized according to whether there was drug resistance (posttreatment marrow remained hypercellular with a predominance of leukemic cells), relative drug resistance (posttreatment marrow became hypocellular with persistence of leukemic cells), or aplastic marrow (posttreatment marrow was hypocellular with no recognizable leukemic cells).

Although the remission rate did increase during dose escalation from level 4 to 5 (4%–16%), there was no further improvement on escalation to dose level 6 (17%). Overcoming absolute or relative drug resistance was not achieved by the increased doses of drugs, with 57%, 42%, and 54% of the patients showing drug resistance at dose levels 4–6, respectively.

Remissions, once obtained, were relatively durable. The duration of remission was evaluable in 12 of 13 patients with AML (one patient underwent allogeneic bone marrow transplantation while in first remission) and ranged from 14 to 54 weeks (median, 28). All patients but two achieving CR received maintenance chemotherapy as outlined above. The unmaintained remissions lasted 14 and 36 weeks. The single remission in a patient with ALL lasted only 7 weeks and was the only remission achieved below dose level 4. The 13 responding patients survived a median of 45 weeks from entry in the protocol (range, 19–83 weeks).

Toxicity

The major toxic effects included myelosuppression, hyperbilirubinemia, stomatitis, diarrhea, and cardiac dysrhythmias. The expected myelosuppression was universal, with all patients requiring platelet and rbc transfusions and all developing absolute agranulocytosis. The only patient below dose level 4 who developed significant nonmyeloid toxic effects was the same patient with ALL who achieved CR. Toxic effects included transient but severe hyperbilirubinemia (31 mg/dl) and intractable nausea, despite antiemetics.

At dose levels 4–6, some degree of hepatic and gastrointestinal toxicity was common (table 4). Bilirubin elevations > 3 mg/dl were noted in 25% of the patients, but were transient and not associated with evidence of hepatocellular damage. Hyperbilirubinemia was not associated with hepatic coma in any patient. At dose levels 4–6, some degree of nausea and vomiting occurred in all patients, stomatitis occurred in 46%, and diarrhea occurred in 58%. The occurrence of more significant hyperbilirubinemia and gastrointestinal toxic effects is presented by dose level in table 4.

Three patients developed nonfatal cardiac dysrhythmias while receiving amsacrine. In one patient the dysrhythmia was atrial flutter requiring cardioversion on the second day, while a second patient had intermittent atrial fibrillation which responded to digitalization. In both of these patients the serum potassium was > 3.5 mEq/L during treatment, and the second patient received a second 4-day course of amsacrine without difficulty. In the third patient atrial tachycardia developed 18 hours after the last dose of amsacrine at a time when the serum potassium was 3.1 mEq/L.

One patient developed acute and lethal cardiac decompensation associated with cardiomegaly, pulmonary edema, and cardiogenic shock 17 days after receiving a second course of 5-azacitidine and amsacrine at the fourth dose level. This patient was a 22-year-old male in first relapse of ALL. He had previously received 300 mg/m^2 of doxorubicin, but at the time of entry in this protocol had a cardiac ejection fraction of 54%. An autopsy was not performed.

TABLE 3.—Clinical outcome by dose level*

	Dose level					
	1	2	3	4	5	6
Evaluable for response	3	3	2	26	19	52
Absolute drug resistance	3			3	3	8
Relative drug resistance		2	1	10	5	20
Aplasia						
Death		1		4	2	4
Leukemia				4	4	9
PR				1	2	2
CR			1	1	3	9

* Values = No. of patients.

TABLE 4.—Nonhematologic toxicity related to 5-azacitidine and amsacrine*

Toxic effect	Dose level 4	5	6
Hyperbilirubinemia (mg/dl)			
3.1-7	18	16	13
> 7	13	5	13
Stomatitis			
Mild to moderate	30	17	23
Cannot eat	15	26	17
Diarrhea			
Mild to moderate	26	30	29
Hemorrhagic	—	—	6

* Values = % of patients evaluable for toxicity.

DISCUSSION

The remission rate achieved by this combination regimen was not superior to previous experience using 5-azacitidine (6-8) or amsacrine (1-5) as a single agent. Also, we could not confirm the high remission rate obtained in seven of 12 relapsed AML patients reported by Kahn et al (10) using amsacrine and 5-azacitidine concurrently, each as a 30-minute infusion, at a dose of 150 mg/m^2/day for 5 days. It is unknown whether the difference in remission rate between the two protocols can be attributed to the slight differences in total dose of drugs given, the major differences in drug administration schedule, or the fact that the study reported by Kahn et al was from a single institution and involved a limited number of patients. The contribution of this latter factor may be clarified by a current Eastern Cooperative Oncology Group multi-institutional protocol using the regimen described by Kahn et al.

A notable finding in this study was the duration of the remissions once obtained. Our observation of a 28-week median remission duration is in agreement with that previously reported by Kahn et al (10). In contrast, the median remission duration for 5-azacitidine alone has ranged from 15 to 19 weeks (8), while for amsacrine alone it was 8-12 weeks (1-5). In a recently completed SEG primary treatment protocol for adult AML, the inclusion of 5-azacitidine during consolidation followed by intensification with cytarabine and daunorubicin during maintenance gave longer remission durations than in comparable treatment arms not containing 5-azacitidine consolidation (11). The relative contributions of amsacrine and 5-azacitidine to the remission duration in this study are unknown, but there appears to be a synergistic effect when compared to either drug alone.

The toxic effects seen in the combination were not uniformly more severe than when the two drugs have been used as single agents. For instance, the occurrence of hyperbilirubinemia was essentially the same as when amsacrine has been used alone (5), and the incidence of diarrhea was similar to that observed with 5-azacitidine when used alone (8). The occurrence of hemorrhagic diarrhea at dose level 6, although infrequent, suggests this may be a dose-limiting toxic effect. The combination did appear to produce more severe stomatitis than is seen with either drug given alone at the same dose.

The occurrence of cardiac dysrhythmia with amsacrine is well-described (12) and was relatively infrequent in this study. Cardiomyopathy has not been previously reported in adult patients with leukemia, although it has been noted in pediatric patients treated with amsacrine for lymphoid malignancies (13). The single patient with acute cardiac decompensation observed in this study may represent the first case of amsacrine-associated cardiomyopathy in an adult. In the pediatric cases those patients at high risk for developing cardiomyopathy received a total dose of anthracycline plus amsacrine > 900 mg/m^2. Our patient fulfills that criterion, yet many other adults on this and previous amsacrine protocols have also fulfilled the criterion.

In summary, the sequential administration of 5-azacitidine and amsacrine has achieved relatively durable remissions in a minority of patients with resistant or relapsed leukemia. With no improvement in remission rate from dose level 5 to 6, and a slight increase in toxicity at dose level 6, dose level 5 is recommended. However, for reasons noted above, the use of concomitantly administered 5-azacitidine and amsacrine (10) in future protocols may be preferable. Further evaluation of 5-azacitidine-amsacrine combinations in resistant AML appears to be indicated.

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Phase II Trial of Lymphoblastoid Interferon in Metastatic Malignant Melanoma[1]

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Thirty-three patients with metastatic malignant melanoma, some of whom had received previous chemotherapy, immunotherapy, or radiotherapy, were entered in a trial of three different doses and schedules of administration of lymphoblastoid alpha interferon. The overall response rate was 9%; some patients had long-lasting response and two achieved complete response. There was no clear advantage to any one regimen. Dose-related flu-like syndrome, granulocytopenia, hepatocellular enzyme elevation, and anemia were observed as the most common toxic effects. Interferon deserves further study in the treatment of malignant melanoma. [Cancer Treat Rep 69:813–816, 1985]

Malignant melanoma represents 1% of the malignancies in the United States and is responsible for 65% of the deaths due to skin cancers (1). Three therapeutic approaches to metastatic disease have evolved and include chemotherapy, immunotherapy, and limb perfusion. The chemotherapeutic standard for comparison is dacarbazine, which induced a 21% response rate in 1379 patients compiled from 18 series (1). Reports of combination chemotherapy with response rates up to 50% have generally been difficult to reproduce and failed to provide demonstrable survival advantage over single agents (2). Immunotherapy with bacillus Calmette-Guérin (BCG) or methanol-extracted residue (MER) administered either intralesionally or systemically can produce regression in skin lesions in approximately 21% of the patients and rarely in parenchymal disease (2). Perfusion techniques require limb-localized disease. With melphalan and hyperthermia, Stehlin et al (2) obtained an 80% response rate for limb-localized unresectable disease. Improvement in treatment for metastatic melanoma requires identification of new agents or new concepts of therapy.

Genetic engineering has allowed the use of interferon as antineoplastic therapy by making large quantities of purified interferon available for investigational use. Several trials in melanoma have demonstrated that interferon has activity against this disease (3–10). We undertook a phase II trial of interferon to determine the relative efficacy of high, intermediate, and low doses in a study format in which a number of the patients were crossed over to a second dosage and schedule.

PATIENTS AND METHODS

Between September 1982 and December 1983, 33 patients (22 male, 11 female) were entered in a study of lymphoblastoid interferon in histologically confirmed and objectively measurable metastatic malignant melanoma. Criteria for eligibility included at least a 10-day interval after surgery and a 30-day interval after radiotherapy or chemotherapy prior to initiation of interferon. Intercurrent medical problems had to be stable. Patients with symptomatic cardiovascular disease were excluded. All patients entered had a life expectancy of > 3 months. Index metastases had to be objectively evaluable by direct measurement in skin or nodal disease or by computerized tomography, sonogram, liver scan, or plain film in parenchymal disease. Brain metastases eliminated any patient from consideration for enrollment and except in the case of one responding patient, from further interferon therapy. Hematologic requirements included wbc count $> 3000/mm^3$, lymphocyte count $> 1500/mm^3$, platelet count $> 100{,}000/mm^3$, and hematocrit > 25%. Normal liver and adequate kidney functions (creatinine < 2.0 mg/100 ml) were specified.

Informed consent was obtained from all patients. No

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patient had had previous interferon therapy and all patients had an Eastern Cooperative Oncology Group performance status (PS) of 0 or 1, with the exception of one patient with a PS of 2 at the start of therapy. The mean age was 44 years (range, 22–76). All patients had undergone previous surgery for primary excision and for confirmatory biopsy of recurrent disease. Previous chemotherapy either in the adjuvant or advanced disease setting had been given to 12 patients, previous radiotherapy had been given to four patients, and previous immunotherapy with BCG or MER had been given to three patients. Twenty-six patients had skin or nodal disease and 20 had parenchymal disease of lung, liver, or kidney. All patients were evaluable for toxicity in all courses of therapy and were evaluable for response in all cycles of therapy except two. The two unevaluable patients pursued rapidly declining courses and died after having received < 1 week of interferon on the various schedules. Response was defined as a 50% decrease in the sum of the products of the perpendicular diameters of measurable disease without the appearance of new lesions.

There were three schedules of human lymphoblastoid interferon (Wellferon) administration. Patients treated with Schedule A were injected im with four cycles of 15×10^6 units/m^2/day of interferon on Days 1, 3, 5, 8, 10, and 12, followed by a 1-week rest period. On Schedule B, im injection of an intermediate dose of 5.0×10^6 units/m^2/day once weekly was administered. Schedule C prescribed im injection with a low dose of 0.5×10^6 units/m^2/day on weekdays for 6 weeks. Schedule A was chosen on the basis of phase I data as a maximum tolerated dose/schedule (11). Schedule B was intended to be immunomodulatory and well-tolerated; it was chosen on the basis of our phase I data with a Cantell-type natural mixture of alpha interferons. It was the original intention to compare the two. Because of a small goal for accrual of patients, additional information was sought by crossing over patients with disease progression to the other arm. Midway through the study it became clear that Schedule B was not immunomodulatory in terms of the index assay of natural killing. This schedule was supplanted by Schedule C on the basis of phase I data from Duke University (11). After this change, patients received Schedule C initially and were crossed to Schedule A during disease progression. Initially, then, patients assigned to Schedule A were crossed to B during disease progression and B were crossed to A. The final 14 patients assigned initially to Schedule C were eligible to cross to A during disease progression.

De-escalation of dose or termination of interferon therapy was required with the occurrence of World Health Organization grade III toxicity (SGOT > 300 mg/100 ml or more than twice the pretreatment level; wbc count < 2000 mm^3, granulocyte count < 1000/mm^3; PS > 3, etc). Patients were examined and had cbcs and blood chemistries done at least monthly. Imaging studies of measurable disease were performed at least monthly, with crossover at disease progression. Patients with responses were continued on therapy until a partial response had not improved for 1 month or until relapse in the case of complete response. Patients with stable disease received four cycles of therapy.

RESULTS

In all arms of the study 52 courses of interferon were administered, with two courses judged too short to be evaluable. Overall results include a complete remission of skin disease in one patient and liver disease in another. One patient had partial remission and two had mixed response. The remaining courses of therapy were discontinued if a patient had stable or progressive disease.

Of the 25 patients treated with the high dose (Schedule A), 12 after crossover from Schedule B or C, one was unevaluable for response due to rapid disease progression. One complete responder had complete disappearance of skin disease and remained disease-free for 10 months after apparent human lymphoblastoid interferon hypersensitivity forced discontinuation of interferon during the 4th month of therapy. Two patients in whom disease progression had been documented on low-dose treatment had mixed response, with shrinkage or disappearance of some skin nodules while others enlarged. A patient with liver disease who was injected for 2 months had disease progression after 7 months of stable disease.

Of the 12 patients treated with intermediate-dose weekly interferon injection during Schedule B, six after crossover from Schedule A, one was unevaluable for response because of rapid disease progression and general deterioration after one dose. A single patient withdrew voluntarily after 2 months of therapy with stable skin and pelvic disease. No partial or complete responses were observed with this regimen. None of the four patients crossing to Schedule A responded to the more intensive regimen.

All of the 14 patients treated with the low dose prescribed by Schedule C were evaluable for response; none had received prior interferon. One patient developed seizures resulting in discovery of a CNS metastasis while liver metastases documented by biopsy were responding to therapy. After brain irradiation, interferon injections were resumed and the patient continues to maintain a complete remission 15 months after initiation of therapy. Remission was documented by normalization of mass lesions on computed tomograms of the head and on sonograms of the liver. A patient with skin and lung disease had a partial response which persisted during 3 months of therapy. Two pa-

tients with skin disease, both treated for 2 months, had stable disease for 3 and 4 months.

The median survival of the cohort of 33 patients was 32 weeks. Fifteen patients are alive at this writing. Six patients (18%) survived for > 1 year. Of these patients, five survive at this writing.

The two patients who had complete response were previously untreated except for surgery. The patient who had partial remission and three of the patients with stable disease had had previous chemotherapy or immunotherapy. The two patients with mixed response had had no previous therapy for melanoma other than surgery.

Interferon toxicity has been well-documented (12,13). Typical symptoms of fever, chills, nausea, and fatigue were dose-related and mandated dose reduction in three patients. Neutropenia required dose reduction in a single patient, with an absolute granulocyte count $< 1000/mm^3$. All patients requiring dose reduction were on Schedule A. Mild to moderate hepatocellular enzyme elevation was noted in 11 patients. Three patients developed anemia: one due to vaginal bleeding of unclear etiology (coagulation parameters were normal); one due to bleeding from autopsy-proven gastric metastases; and the other of unknown cause. Two patients manifested an increase in pre-existing thrombocytosis of > 1 million platelets/mm^3 without hemorrhagic or thrombotic sequelae. Atrial fibrillation was noted during therapy in one patient who later had autopsy documentation of myocardial metastases. The patient whose skin metastasis completely disappeared during therapy developed a chronic urticarial skin reaction. The reaction was documented by a strongly positive immediate reaction to intradermal challenge with 100,000 units of interferon. Biopsy of the lesions, which persisted for weeks, showed dermal edema with an infiltrate of eosinophilic polymorphonuclear cells, suggestive of urticaria. Anti-interferon antibody titers were not obtained.

There were five patients who died within 1 month of receiving their last injection of interferon. Four had rapidly progressive disease and discontinued interferon 22–26 days prior to death. They were felt to have died of progressive metastatic malignant melanoma without evidence of interferon toxicity. One patient on Schedule C had an interferon injection on the day of death. Serial chest x-rays revealed rapidly enlarging pulmonary nodules and a massive pleural effusion; the patient died in respiratory failure.

DISCUSSION

Reports of median response rates of 13% (range, 0%–22%) in patients with melanoma treated with interferon in a variety of doses and schedules have been published (3–10). The overall response rate in this series was 9%. However, one patient with extensive visceral disease and one with minimal cutaneous disease achieved prolonged complete remission, which persists to the present.

All patients were monitored extensively for immunologic changes. A detailed analysis will be published elsewhere. Natural killing was not augmented in a consistent fashion on any schedule, but an activity of tumor growth inhibition mediated by OKTIO+ mononuclear cells was significantly augmented on Schedules A and B but not C. The responders on Schedule C did individually show increased immune activity in assays performed. Most strikingly, the single patient with visceral disease and complete response showed the most drastic augmentation of natural killing of any of the nearly 100 patients treated with interferon whom we have studied immunologically.[4] These observations raise the possibility that improved knowledge of the relationship between natural antitumor immunity and interferon might permit more rational selection of patients for treatment and thereby improve the response rate of melanoma to interferon.

No advantage of one schedule over the others could be ascertained due to the low response rates observed. Responses were seen on low-dose therapy; this indicates that treatment to toxicity is not necessary for response. The continued response of the patient in whom CNS metastasis was manifested and irradiated during the protocol shows that CNS disease need not be an exclusionary criterion in these patients, despite the documentation of a blood-brain barrier to interferon (14–16). This study confirms modest activity of interferon in melanoma and adds support to continued investigation of varied schedules and dosages of interferon administration as well as possible combinations with cytotoxic antineoplastic drugs or radiotherapy.

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Phase II Study of Human Lymphoblastoid Interferon in Patients With Advanced Renal Carcinoma[1,2]

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Twenty-two patients with measurable metastatic renal carcinoma were treated with human alpha lymphoblastoid interferon (Wellferon) (3 million units/m² of body surface area) im three times a week for 6 weeks; 21 were evaluable for response and toxicity. One patient had a partial regression of disease lasting 39 weeks and another had a minor response. Six patients had partial or minor response in one area but no change in the disease elsewhere (mixed response); they were classified as having stable disease. An additional seven patients had no change in measurable disease and in six the disease progressed. The tumor regressions by metastatic site were four of 14 patients, pulmonary; three of four, soft tissue; one of nine, bone (? soft tissue); none of three, mediastinal; none of four, renal; none of five, liver; and none of ten, other abdominal sites of tumor. The side effects were fever (101°F–103°F), chills, malaise, anorexia, minor (10%–20%) decrease in performance status, and weight loss. We concluded that interferon had some activity against metastatic renal carcinoma, although clinically useful responses were quite infrequent. While lung metastases appeared to be more responsive than those at other sites, careful examination of the data suggests that this simply reflects differences in the bulk of the metastatic disease. Smaller metastases appear more likely to respond than bulky metastases. [Cancer Treat Rep 69:817–820, 1985]

Patients who have distant metastases from renal carcinoma have a poor prognosis, with a median survival of approximately 6–8 months (1). Those patients who have metastases at the time of diagnosis or develop them within 6 months have a significantly shorter survival than those who develop metastases > 2 years after nephrectomy. Many forms of systemic therapy have been tried in the treatment of metastatic renal carcinoma. In a large number of patients, hormonal agents produced a temporary benefit in only about 5% (2). Single chemotherapeutic agents have produced similar results, an overall response rate of approximately 5%–10% (2). Chemotherapy combined with hormonal agents or multiagent chemotherapy has produced tumor regression in 7% and 18% of evaluable patients, respectively (2). The responses were transitory and did not significantly affect the survival of the cohort population.

Rare reports of spontaneous regression of metastatic renal carcinoma have provided support for the speculation that the host's immunity may play a role in the control and treatment of this disease. A variety of immune therapy programs such as BCG vaccination (3), immunization with autologous cells (4,5) and antigens (6–8), etc, have been tried; although they gave "promising results," no confirmed antitumor responses of ⩾ 20% have been achieved. Therefore, when sufficient quantities of alpha interferon became available for clinical trials, renal cancer became a natural target for research. Phase I evaluation of human lymphoblastoid alpha interferon in patients with cancer demonstrated a sustained enhancement of natural killer cell activity when low-dose lymphoblastoid interferon was administered im three times a week over 5 weeks, whereas high doses of interferon suppressed natural killer cell activity (9). Monocyte functions, immunoglobulin levels, complement levels, and skin reactivity were not significantly affected by any interferon dose tested. With a dose escalation schedule, the maximum dose which could be tolerated by these cancer patients was 15 million units/m² or a total of 135 million units/m² over 5 weeks. At the highest doses, changes

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in cognitive and behavioral functions were quite disturbing to the patients and their families but they were reversible within 7-10 days after cessation of interferon therapy. Toxic effects such as severe depression, malaise, etc, necessitated the discontinuation of treatment. Based on the findings of the phase I trial, a dose of 3 million units/m^2 im three times a week for 6 weeks was selected for phase II study of the treatment of advanced renal cancers. That experience serves as a basis of this report. The preliminary data were presented previously (10).

MATERIALS AND METHODS

Twenty-two patients with measurable metastatic renal carcinoma were entered in an interferon treatment trial between December 1982 and July 1983. To qualify for the trial, patients were required to have bidimensionally measurable disease (not previously radiated), Karnofsky performance status of 60% or better, no prior systemic chemotherapy or hormonal therapy for at least 4 weeks (nitrosourea, 6 weeks), and no residual adverse effect from prior therapy. Patients were not accepted for the trial if the only manifestation of disease was pleural effusion, bone lesions, brain metastases, or previously irradiated lesions. Patients with metastatic liver disease were acceptable candidates if the disease was measurable by a liver computerized tomographic (CT) scan.

The human alpha lymphoblastoid interferon (Wellferon) used in the trial was prepared by the Wellcome Foundation, Ltd, from Sendai virus-stimulated Namalva cell lines. Patients were treated with alpha interferon at a dose of 3 million units/m^2 of body surface area im three times a week (Monday, Wednesday, and Friday) for 6 weeks. Evaluation was carried out every 2 weeks. All tumor measurements, including CT scans of abdominal masses, were repeated after 6 weeks of therapy. Patients who were responding to treatment continued with the same schedule beyond the initial 6 weeks until no further regression of the disease could be induced. Patients with stable disease at 6 weeks were randomized to either maintenance therapy with interferon (3 million units/m^2) once a week or no maintenance therapy. Patients whose disease progressed during the first 6 weeks of therapy or on maintenance therapy or observation were taken off the study.

Complete response was defined as the disappearance of all measurable disease for a minimum of 4 weeks. Partial response was defined as a decrease of $\geq$ 50% in the sum of the products of the two largest perpendicular diameters of the measurable lesions, lasting for a minimum of 4 weeks, and no appearance of new lesions. Minor response was defined as a decrease of $<$ 50%, but $>$ 20%, and no new lesions. Stable disease was defined as no significant change in the size of the lesions, compared to pretreatment measurements. Several patients with a mixed response (partial or minor response in the chest and no change in the abdomen) were also considered to have stable disease.

The patient population consisted of 16 males and six females, with a median age of 59.5 years (range, 37-78). The median time from diagnosis to interferon treatment was 392 days (range, 7-3589). Prior treatment consisted of nephrectomy in 18 patients, chemotherapy in 14, hormonal therapy in nine, and radiation therapy in seven. Interferon was the first systemic treatment in four patients (two with and two without prior nephrectomy). The cancer was documented in the kidney in four patients, liver in five, abdomen (not in kidney or liver) in ten, lung in 14, mediastinum in three, bone in nine, and soft tissue in four.

RESULTS

Of the 22 patients entered in the trial, 21 were evaluable for response and toxicity. A 56-year-old patient with a history of myocardial infarction and severe angina died from an apparent myocardial infarction within 24 hours after being placed on the protocol. Between the time of the interferon injection and the cardiac arrest, the patient did not appear to have any side effects from the interferon which could have contributed to cardiac decompensation and the patient's death. He was considered to be inevaluable. Of the 21 evaluable patients, 18 completed the 6-week trial of interferon. Three patients were taken off the study after 4 weeks of treatment because of disease progression. One patient was taken off the study after 5 weeks because of mental confusion and extreme weakness despite a 50% dose reduction. Seven patients were randomized to receive weekly maintenance therapy.

Of the 21 evaluable patients, one had partial regression of disease lasting 39 weeks and another patient had minor response. Six patients had partial (one patient) or minor (five) response in one area, but no change in the disease elsewhere (mixed response), and they were classified as having stable disease. An additional seven patients had no change in measurable disease and six had disease progression.

The single partial response occurred in a patient with pulmonary metastases who had had nephrectomy. In this patient, there originally was a complete disappearance of smaller lesions (1.5 $\times$ 1.5 cm, 2.8 $\times$ 4.1 cm), nearly complete regression of a larger lesion ($>$ 5 cm), and disappearance of the pulmonary effusion.

An additional patient had an 80% regression of a neck mass, but the renal mass shown by abdominal CT scan remained unchanged 16 weeks after the beginning of the trial. He was hospitalized for pulmonary emboli and subsequently died from CNS bleeding while being treated with anticoagulant agents.

Another patient of interest was a 63-year-old man with stable disease after 6 weeks of therapy. He showed a dramatic regression of some of the pulmonary metastases

during the maintenance treatment. However, his abdominal CT scan remained unchanged and he was classified as having stable disease. The smaller lung lesions responded, while the bulkier ones remained unchanged.

The correlation of the site of disease with the response (regression) is shown in table 1.

The side effects of this regimen were mainly constitutional, including fever (101°F–103°F), fatigue, malaise, anorexia, weight loss, 10%–20% drop in performance status, etc.; they were almost universal. An average weight loss of 3.05 kg (range, −8 to +1) was observed by the end of the 6-week trial. This represented a mean weight loss of 3.7% of the pretreatment body weight (range, -8.8% to +1%). One patient, a 57-year-old woman, developed profound weakness and severe hallucinations associated with electroencephalogram changes (diffuse slowing of activity) while receiving the treatment, but she nevertheless completed the 6-week trial.

DISCUSSION

We were impressed with the observation that while some metastases showed partial to complete regression, others were stable or even progressed simultaneously. Pulmonary or soft tissue metastases appeared to respond better to interferon than primary tumor. Yet, while we saw excellent regressions of small lung lesions, there was no response in large lesions in the same patients. A similar phenomenon was observed in other tumor locations, suggesting that responses may correlate better with the bulk of the disease than with the specific location of the metastatic deposits. This observation, however, requires further confirmation. In our patients, abdominal disease was always bulky and we saw no objective regression of it. Metastatic lesions in the chest and soft tissues ranged from minimal (≤ 2 cm in diameter) to bulky (≥ 5 cm in diameter). Patients with an unresected primary tumor were more likely to have bulky abdominal disease and less bulky pulmonary and soft tissue metastases; this fact, combined with our observation that smaller lesions responded better than larger lesions, could explain why responses were seen only in the lungs and soft tissues.

The response of pulmonary metastases to interferon

TABLE 1.—Lymphoblastoid interferon in renal cancer: correlation of site of disease with response

Location of disease	No. of patients	
	Total	Responding
Renal	4	0
Liver	5	0
Other abdominal site	10	0
Lung	14	4
Mediastinum	3	0
Bone	9	1*
Soft tissue	4	3

* Bone metastasis extended into the soft tissue.

treatment has been reported previously (11). Three million units/day of partially purified human leukocyte alpha interferon given to 19 patients with metastatic renal carcinoma resulted in five partial remissions (26%) of the pulmonary lesions. All 19 patients had previously undergone nephrectomy, with resection of the primary tumor. Of the 19 patients, 17 had lung metastases (nine had additional skeletal metastases and one had liver metastases) and two had retroperitoneal lymph node involvement only. This group of patients also had renal carcinoma of a "less aggressive" type. Only seven patients had metastases at the time of diagnosis or developed them within 6 months of diagnosis.

Another group observed five partial regressions (four in the lung and one in the renal bed documented by CT scan) among 33 patients with renal carcinoma who were treated with lymphoblastoid interferon (5 million units im 3 times a week for 24 weeks) (12). The median time from treatment to response was 88 days (± 75 days). An abdominal CT scan was not routinely used in the pretreatment evaluation. Although not directly stated, it appeared that the patients who achieved partial remission had had prior nephrectomy and removal of the primary tumor. An additional 23 patients were treated with a 10-day escalating regimen of 3, 5, and 10 million units/day im followed by 20 million units/m^2 im × 7 days, repeated every 21 days for seven courses. One complete and three partial responses were observed. The investigators also treated 13 patients with desensitization with 5 million units/m^2 iv on Day 1 and 10 million units/m^2 on Day 2, followed by 50 million units/m^2 iv × 3 days, repeated every 21 days for three courses. One partial response was reported. No conclusions about the relation of tumor response to size were drawn by the investigators.

One cooperative group study reported five (15%) partial responses among 33 patients with advanced renal carcinoma treated with lymphoblastoid alpha interferon (3 million units/m^2 on Day 1, 5 million units/m^2 on Day 2, 10 million units/m^2 on Day 3, and 20 million units/m^2 on Days 4–10, repeated every 21 days) (13). The time to response was 107 days (range, 48–169), and the duration of response was 30–176 days. Of the 33 patients, 11 had leukopenia (< 2000 cells/mm^3), 16 had twofold increase in baseline hepatic enzymes, 11 had performance status of 3 or 4, and ten had greater than or equal to a two-level change in performance status. Treatment was interrupted or doses were reduced in 23 of 33 patients during the first cycle of therapy. Only nine of 33 patients completed two 10-day cycles at the full dose.

In a randomized trial of recombinant alpha II interferon in which 26 patients were randomized to receive either 30 million units/m^2 iv × 5 days every 2–3 weeks or 2 million units/m^2 sc three times a week, there was one partial response in the high-dose arm (14).

Differences in the response rates reported by different centers are probably not biologically significant and may

be related to the bulk of the metastatic deposits, heterogeneity of the tumor cell, and variable response rates to interferon. For example, of the 25 successful renal cell cancer cultures studied in a clonogenic assay and exposed to interferon, 11 showed sensitivity (> 60%) and 14 showed resistance (< 60% reduction in colony growth) to interferon (15). Seven patients, four with sensitive and three with resistant in vitro lines, were treated with 3 million units of interferon. The four patients with renal tumor showing a > 60% decrease in colony formation to 100 units/ml responded, with two partial responses and two minor responses, while the three patients with tumors showing < 60% in the colony formation had progressive disease. Larger studies are needed to document whether in vitro studies are useful in selecting patients with high probability of a beneficial response to interferon.

The preferential response to interferon of distant metastases such as lung metastases could argue in favor of removing the primary lesion in patients who present with metastases at the time of diagnosis. However, the low response rate to interferon and the lack of evidence that removal of the primary renal tumor significantly affects the results of systemic therapy make such a recommendation premature.

One criticism of our study may be the short duration of mandatory therapy (6 weeks) before evaluation and randomization to maintenance therapy or no maintenance therapy. The delayed response in one of our patients while receiving maintenance therapy may support this view. However, in all malignancies where systematic treatment is very effective, responses are usually seen within 3-4 weeks. In any event, patients showing any sign of regression were continued beyond the 6-week period in our study. Although we had very few patients in the study, the patients randomized to maintenance therapy did not appear to do better than those with no maintenance therapy. Another possible criticism is that the dose chosen to optimize immune responsiveness and limit toxicity may be too low to exploit the growth-inhibitory properties of interferon. This was investigated in a second study, which will be the subject of a future report. Preliminary data, however, did not show better responses at higher doses.

In summary, we can conclude that interferon had some activity against metastatic renal carcinoma, although clinically useful responses were quite infrequent. While lung metastases appeared to be more responsive than those at other sites, careful examination of the data suggests that this may reflect the differences in the bulk of the metastatic disease. Metastases with a smaller volume appear more likely to respond than bulky metastases. Toxicity was achieved in virtually all patients.

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Cisplatin, Vinblastine, and Bleomycin in the Treatment of Metastatic Melanoma: A Phase II Study of the Southeastern Cancer Study Group[1,2]

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Fifty-one patients with metastatic melanoma were treated with cisplatin, vinblastine, and bleomycin. Of the 50 evaluable patients, 11 (22%) achieved an objective response, including three complete (6%) and eight partial (16%) responses. Four of the 11 responding patients had previously received dacarbazine; the remaining patients had received no prior chemotherapy. Responses were noted in cutaneous and lymph node sites as well as visceral metastases. However, with one exception, all responding patients with visceral involvement had lung metastases. Response durations were brief and toxicity was substantial. Nadir leukocyte counts $< 0.5 \times 10^9$/L occurred in 28% of the patients. Debilitating neurotoxicity, primarily paralytic ileus, and severe nausea and emesis were experienced by 24% of the patients. The combination of cisplatin, vinblastine, and bleomycin is not sufficiently beneficial to warrant its use in metastatic melanoma. [Cancer Treat Rep 69:821–824, 1985]

Metastatic melanoma is notoriously difficult to treat. Consequently, the report of Nathanson et al (1,2), which indicated that 47% of patients with melanoma had responded to a regimen consisting of cisplatin, vinblastine, and bleomycin (PVB), was greeted with considerable optimism. Responses were observed in patients with cutaneous and lymph node disease as well as in some with visceral metastases. Subsequently, several groups including the Southeastern Cancer Study Group (SECSG) undertook confirmatory studies to define the activity of PVB and its role in the management of metastatic melanoma.

MATERIALS AND METHODS

Between March 1981 and July 1983, 51 patients with metastatic melanoma were entered in this study. To be eligible, patients had to have measurable disease and a Karnofsky performance status > 40%. There was no age limitation. Patients with brain metastases were ineligible as were patients previously treated with any chemotherapy other than dacarbazine (DTIC) or immunotherapy. All patients were to be considered eligible for response and toxicity evaluation regardless of the number of cycles of PVB received.

Treatment consisted of cisplatin (50 mg/m^2 iv) administered in 1 L of 5% dextrose in normal saline without mannitol, vinblastine (12 mg/m^2 iv), and bleomycin (30 units iv). Cisplatin and vinblastine were administered every 3 weeks, for a total of three cycles, while bleomycin was given weekly for 9 weeks. Initial staging consisted of physical examination; cbc and platelet count; liver enzyme determination; chest x-ray; liver-spleen scan, abdominal computerized tomographic scan, or hepatic ultrasound; bone scan; and bone marrow biopsy if clinically indicated. Patients were restaged at the completion of three cycles of PVB by repeating all initially abnormal studies. Patients achieving a complete or partial response continued receiving cisplatin and vinblastine without bleomycin every 3 weeks until disease progression was documented or treatment-related toxicity prohibited further therapy.

Complete response (CR) was defined as the clinical resolution of all evidence of disease for a minimum of 4

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weeks. Partial response (PR) was defined as a > 50% decrease in the sum of the products of the largest bidimensional diameters of all measurable lesions. Stable disease was defined as a < 50% reduction in tumor size without the appearance of any new lesions. Progressive disease was defined as an increase of > 25% in the size of any measurable lesion or the development of any new lesion.

RESULTS

Patient characteristics are shown in table 1. All patients but one of the 51 entered in this study were considered evaluable for response. One patient with brain metastases was improperly registered. Since no treatment was administered, this patient was considered nonevaluable. However, there were two early deaths due to disease progression, two toxic deaths prior to completing the initial cycle of PVB, and five patients who refused further treatment after the initial course of PVB. All nine patients were judged to have had progressive disease. Thus, of the 50 evaluable patients, three (6%) achieved complete response and eight (16%) achieved partial response, for an overall response rate of 22% (95% confidence limits, 11%-33%). Nine patients (18%) experienced stable disease, while the remaining 30 patients (60%) had disease progression during PVB. Two of the three patients achieving CR had lung metastases with or without lymph node disease and one had isolated lymph node involvement. One patient achieving CR had received prior DTIC. Seven of the eight patients achieving PR had either pulmonary metastases, lymph node disease, or both. One patient with PR had liver and lung involvement. Five patients with PR were previously untreated. The response rate was approximately the same in both sexes (22.5% in males vs 18% in females). The durations of response (determined from time of restaging) were brief, lasting 2, 5, and 5+ months in patients with CR and 2-10 months in patients with PR (median, 3.5 months). Overall median survival (calculated from time of diagnosis) was 5 months (range, 1-14), with responding patients surviving 4-14 months (median, 7). Patients with stable disease survived a median of 9 months (range, 2-11).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	51
Sex	
Male	40
Female	11
Median age in yrs (range): 47 (19-71)	
Performance status	
70%-100%	47
40%-60%	4
Previous chemotherapy	12
No previous chemotherapy	39
Sites of metastases	
Soft tissue only	10
Visceral ± soft tissue	41

TABLE 2.—Hematologic toxicity

Toxicity grade*	Nadir counts $\times 10^9$/L	No. of patients
	Wbc count	
0	> 2.0	13
1	1.5-1.9	4
2	1.0-1.5	8
3	0.5-1.0	11
4	< 0.5	14
	Platelet count	
0	> 130	43
1	90-130	5
2	50-90	1
3	25-50	0
4	< 25	1

* SECSG criteria.

Although most patients had received no previous chemotherapy, myelosuppression was the primary toxic effect encountered (table 2). Moderate leukopenia (nadir wbc count < 2.0×10^9/L) was observed in 74% of the patients and severe leukopenia (wbc count < 0.5×10^9/L) was observed in 28%. Thrombocytopenia was relatively uncommon, with only one patient (2%) experiencing a platelet count nadir < 50×10^9/L. There were no episodes of life-threatening hemorrhage.

The major nonhematologic toxic effects are summarized in table 3. Severe nausea and emesis (ie, not controlled with antiemetics) was observed in 24% of the patients. One patient developed uremia and four experienced a mild to moderate increase in serum creatinine to > 2.0 mg/dl. Paralytic ileus was common, occurring in 12 patients (24%). Concomitant neurotoxic effects included dysphagia in two patients (4%) and leg weakness in four (8%). All neurologic abnormalities resolved with either a reduction or discontinuation of vinblastine. Bleomycin-induced pulmonary toxicity was documented in one patient and suspected clinically in a second. A ⩾ 20% weight loss was noted in two patients.

DISCUSSION

Metastatic melanoma has proven particularly re-

TABLE 3.—Nonhematologic toxicity

Toxicity	No. of patients
Renal (creatinine > 2.0 mg/100 ml)	5
Nausea/emesis*	12
Documented infection	6
Neurotoxicity†	12
Pulmonary toxicity	2

* Uncontrolled with antiemetics; SECSG, grade 3.
†Ileus, weakness, dysphagia.

TABLE 4.—Response according to sex, metastatic site, and prior chemotherapy

	No. of patients						
	Total	CR	PR	Stable disease	Progressive disease	Not evaluable	*P* value*
Total	51	3	8	9	30	1	—
Male	40	3	6	8	22	1	> 0.5
Female	11	—	2	1	8	—	
Visceral metastases	38	2	6	8	22	—	> 0.5
Soft tissue metastases	13	1	2	1	8	1	
No prior treatment	39	2	5	5	26	1	> 0.1
Prior treatment	12	1	3	4	4	—	

* Chi-square analysis.

fractory to systemic chemotherapy (3). Until recently, most chemotherapy regimens studied have attempted to exploit the activity of DTIC in combination with various other agents including nitrosoureas (4). In 1980, Nathanson et al (1) reported that PVB could effect a high percentage of objective responses in patients with metastatic melanoma. Impressively, PRs and CRs were noted even in patients with visceral metastases (2). Most studies have indicated that the site of metastatic disease will have a profound effect on objective response, with cutaneous and lymph node disease having higher response rates than visceral sites of disease (5). Thus, the possible improved activity of PVB sparked considerable interest in the use of this regimen in metastatic melanoma.

Several groups have attempted to corroborate the findings of Nathanson et al (1). In a study by Creagan et al (6), only two of 18 patients (11%) experienced an objective response. Bajetta et al (7) reported an objective response in eight of 29 evaluable patients (28%), including three CRs. However, all responses occurred in patients without visceral metastases. York et al (8) noted no objective responses in 20 evaluable patients given PVB. Similarly, the Canadian National Cancer Institute noted only 12 objective responses (19%) in 64 evaluable patients (9). Although objective responses were observed in visceral as well as soft tissue metastases, this group concluded that the severity of treatment-induced toxicity along with the brevity of response durations abrogated any potential benefit of PVB as front-line therapy for metastatic melanoma. Similar conclusions were drawn by Creagan et al (6) and Bajetta et al (7).

Our data confirm the results of the previously mentioned negative studies. Although objective responses were noted in 11 patients (22%), including three CRs, all were of brief duration. Like the Canadian National Cancer Institute, we noted objective responses in visceral and soft tissue sites (table 4). However, only one patient with liver metastases experienced an objective response. The remaining responding patients with visceral metastases all had pulmonary nodules. Survival was brief regardless of treatment response and toxicity was substantial, with about 25% of the patients experiencing profound myelosuppression resulting in two treatment-related deaths. Neurotoxicity was also significant and necessitated hospitalization for paralytic ileus in several patients.

The Canadian National Cancer Institute noted a higher response rate in women (30%) than in men (9%). Based on this observation, it was suggested that phase II studies consisting exclusively or predominantly of male subjects might yield misleading negative results. This may be one explanation for the low overall response rate obtained in our patient group, since most of our patients were men. However, the response rates were identical in the males and females in our study. Also, the scheduling of bleomycin and vinblastine administration used in this study differed somewhat from that employed by Nathanson et al (2). While these changes in bleomycin and vinblastine administration may account for the less optimistic results obtained in this study, the previously discussed negative studies all used a regimen of infusional bleomycin and divided doses of vinblastine (8,9). Thus, it appears unlikely that the difference in response rate is due solely to the changes in drug administration.

In summary, PVB can effect a modest objective response rate in patients with metastatic melanoma. However, the short duration of responses coupled with the high level of toxicity indicates that this regimen is no better than treatment with single-agent DTIC.

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High-Performance Liquid Chromatographic and Enzyme-Inhibition Assays of Methotrexate Concentrations in Serum From Patients Receiving High-Dose Therapy: Tight Binding of Some Methotrexate to a Protein That Could Be Dihydrofolate Reductase[1]

H. Iven[2,*] and H. Bartels[2,3]

In the serum of patients receiving high-dose methotrexate (MTX), concentrations of the drug were monitored using both high-performance liquid chromatographic (HPLC) and enzyme-inhibition assays. In samples obtained ≥ 24 hours after drug application, the HPLC assay measured higher MTX concentrations than the enzyme-inhibition assay. In 72-hour samples, the increase was usually > 200%. This difference was not observed in serum spiked with MTX. While the HPLC assay needs sample cleanup, ie, protein precipitation, the enzyme-inhibition assay routinely employs native serum. When MTX was measured in the supernatant with the enzyme inhibition assay, the results equaled those obtained with the HPLC procedure. In patient serum, MTX eluted from a Sephadex G-75 column in two fractions. One had the same retention volume as free MTX and could be assayed directly. The other had a retention volume like dihydrofolate reductase. In this fraction, MTX could only be determined after denaturing proteins. Only small amounts of tightly bound MTX were found in the serum of patients on intermediate-dose therapy (500–600 mg/m^2). The observation that after high-dose MTX therapy part of the MTX in patient serum is strongly bound to a protein which could be DHFR raises the question as to the source and fate of this complex. Furthermore, the present finding has clinical relevance to the extent that the accepted limits for risk of MTX toxicity are based on methods using native serum. However, any procedure employing protein precipitation for sample cleanup may grossly overestimate active MTX serum concentrations, especially in the 72-hour serum samples. [Cancer Treat Rep 69:825–832, 1985]

To reduce the risk of a high-dose methotrexate (MTX) therapy which is potentially lethal, it is mandatory to monitor the MTX concentration in patient serum. Several analytical methods have been reported. These methods may be divided into those using native serum like the radio- and enzyme-immunoassays, the competitive protein-binding assay, the ligand-binding radioassay, the dihydrofolate reductase (DHFR) inhibition assay, and those usually needing sample cleanup before analysis, like the spectrofluorometric and the high-performance liquid chromatographic (HPLC) methods. While the former are very sensitive, a cross-reaction with MTX metabolites may occur. In contrast, HPLC procedures give more specific results, but usually suffer from lower sensitivity. Aherne and Quinton (1) have performed a recent comparison of different techniques.

When we tried to change routine MTX analysis from DHFR inhibition assay to a HPLC procedure, we frequently found higher MTX concentrations in patient serum with the latter method, especially in samples obtained 48 hours after drug application. One of the differences between both methods is the precipitation of proteins prior to HPLC analysis, and we report here experimental results indicating that part of the MTX in patient serum is firmly bound to a protein which may be DHFR.

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METHODS

Serum samples from four patients with osteogenic sarcoma who were receiving high-dose MTX therapy (18–20 g) were taken at the end of the 6-hour MTX infusion period, and 24, 48, and 72 hours after the beginning of the infusion. Serum samples from six patients receiving intermediate-dose MTX therapy (500–600 mg) were obtained for MTX determination 24 and 48 hours after initiation of the 4-hour infusion.

Enzymatic Analysis

MTX serum concentrations were determined with an enzymatic assay using DHFR from bovine liver (2). The assay mixture was prepared daily from stock solutions and contained (final concentrations, mol/L): 0.15 phosphate buffer pH 6.5, 3×10^{-3} sodium azide, 1.2×10^{-4} NADPH (nicotinamide adenine dinucleotide phosphate, reduced form), 14×10^{-3} β-mercaptoethanol, 1.5 g/L of bovine albumin, and 5 U/L of DHFR. Either 0.05 ml of MTX standard ($1.2–4.8 \times 10^{-8}$ mol/L) or 0.05 ml of sample was added to 1 ml of assay mixture. After 5 minutes of preincubation at 37°C, the reaction was started by adding 50 µl dihydrofolate solution, which resulted in a final concentration of 5.6×10^{-5} mol/L. The reaction was followed in a Zeiss PMQ III (Carl Zeiss, Oberkochen, West Germany) recording spectrophotometer equipped with an automatic cell changer and temperature regulation. The linear decline of absorbance during the first 4 minutes was a measure of the enzyme activity. The inhibition of enzyme activity by MTX was linear in the range of the standards ($1.2–4.8 \times 10^{-8}$ mol/L), and all samples, if necessary, were diluted to fall within this range.

HPLC Analysis

To precipitate proteins, 0.8 ml of serum was mixed with 0.4 ml of 0.04 N acetic acid and heated for 5 minutes at 96°C. After centrifugation for 2 minutes at $12,000 \times g$, the clear supernatant, which had a pH of 6.4, was transferred to the HPLC system. It consisted of an automatic injection system (WISP 710B, Waters Associates, Inc, Milford, MA), a Constametric III pump, a variable wavelength LDC spectromonitor III (Laboratory Data Control, Riviera Beach, FL), and a HP 3390A computing integrator (Hewlett-Packard Co, Avondale, PA). The 0.46 × 25-cm analytical column was filled with Shandon-Hypersil-ODS-5-µm and was protected by a guard column (0.46 × 4 cm) filled with the same material. A solvent mixture of 15% methanol in 0.01 M phosphate buffer pH 7 (vol/vol) was used as eluent; the flow rate was 1.2 ml/minute. Chromatography was performed at room temperature; the absorbance was monitored at 313 nm.

Chromatography on Sephadex G-75

Gel filtration chromatography was performed on a 1.5 × 37-cm Sephadex G-75 column, which had an exclusion volume of 20 ml. The eluting solvent was 0.01 M phosphate buffer pH 7 containing 1.5 g/L of bovine albumin and 0.2 g/L of sodium azide. (Without albumin in the buffer, no DHFR activity could be eluted from the column, probably due to adsorption of the enzyme onto the glass surface of the column.) The flow rate was 64 ml/hour, and 2-ml fractions were collected. The absorbance was monitored at 254 nm. In two separate runs, 0.5 ml of solution containing either 200 mU of DHFR from bovine liver or 1.5×10^{-6} mol/L of MTX was applied to the column for reference.

After application of DHFR onto the column, enzyme concentration in the eluate was determined with the same method as described for MTX determination, except that the enzyme was omitted from the reaction buffer and added as the variable.

In a third run, 0.5 ml of patient serum that was obtained 24 hours after high-dose MTX therapy was applied to the column. During this run, MTX in each fraction was determined twice with the enzymatic assay, ie, in the native eluate as well as in the supernatant after protein precipitation.

Equilibrium Dialysis

One milliliter of patient serum in Visking 8/32 dialysis tubing was dialyzed for 16 hours against 1 L of a 0.02 M phosphate-buffered 0.9% saline solution, pH 7.4, at room temperature. The dialysis sac was fixed to the magnet of a magnetic stirrer and rotated in the beaker at approximately 30 runs/minute.

RESULTS

Clinical monitoring of MTX was done with the enzymatic assay, using native patient serum. Results obtained were used for adjustment of leucovorin rescue.

Patients Receiving High-Dose MTX Therapy

Twenty-three serum samples obtained 48 hours following the iv infusion of high-dose MTX to four patients were analyzed by HPLC and enzyme inhibition assays. A close correlation of results was obtained when MTX was measured with the enzyme inhibition assay in the supernatant of the serum after protein precipitation (fig 1). Note that this supernatant was also used for HPLC analysis. The slope of 0.97 indicates equal results by both methods.

Figure 2 shows the correlation of results when MTX was measured with the enzyme inhibition assay both in native serum and in the supernatant (same serum sam-

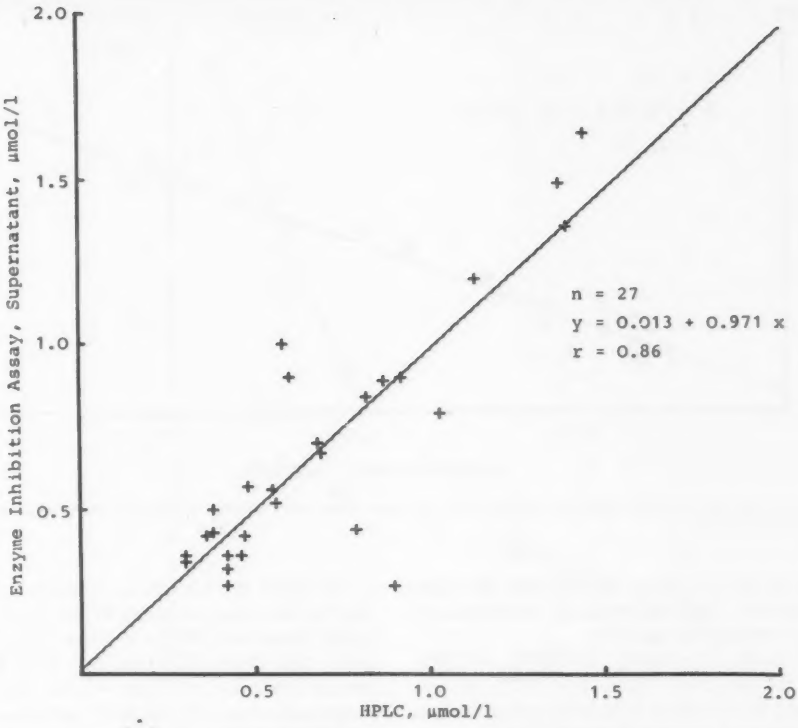

FIGURE 1.—Comparison of results obtained by HPLC and enzymatic assays after protein precipitation (supernatant). Samples from 4 patients obtained 48 hrs following a 6-hr infusion of high-dose MTX.

ples as in fig 1). The slope of 0.376 indicates that in 48-hour serum samples, MTX concentrations in the supernatant differ from those of native serum by a factor of 2.5.

In our hands, the enzymatic assay was more reliable than the HPLC assay, especially with the low concentrations found in patients' serum after 72 hours. Therefore, all further results presented in this paper are those obtained with the enzymatic assay both in native samples and in supernatant.

The MTX concentrations found in the serum of four patients receiving high-dose MTX therapy are shown in table 1, and the mean values of 14 infusions of Patient 1 are shown in figure 3. MTX concentrations in serum at the end of a 6-hour infusion were in the range of 1 $\times$ 10^{-3} mol/L (6-hour value) and were the same in native serum and supernatant. During the next 18 hours (24-hour value), concentrations declined by two to three orders of magnitude. At this time, with a few exceptions, concentrations in supernatant were higher than in native serum. This was always the case in serum obtained 48 and 72 hours after the beginning of the MTX infusion. The increase in MTX concentration after protein precipitation, ie, the difference in concentration between native serum and supernatant (Δ values in table 1), was highest 24 hours after infusion and declined monoexponentially (fig 3). In relative terms, the increase was most impressive after 72 hours, where it amounted to up to 200% of the concentration found in native serum.

Since additional MTX was obviously liberated by protein precipitation, it was of interest to see whether some protein-bound MTX was already present at the end of the MTX infusion (6 hours) and was only masked by the excessive concentration of free MTX. Therefore, from ten high-dose MTX infusions given to Patient 1, 1 ml each of 6-hour serum was dialyzed against 1 L of phosphate-buffered saline for 18 hours. This reduced free MTX concentration in serum by three exponents. However, the MTX concentration found in native serum after dialysis was not different from that found in the supernatant after protein precipitation of the dialyzed samples.

Dialysis was also performed with some of the 24-hour serum samples of Patient 1. In absolute terms, the difference in concentration between native serum (after

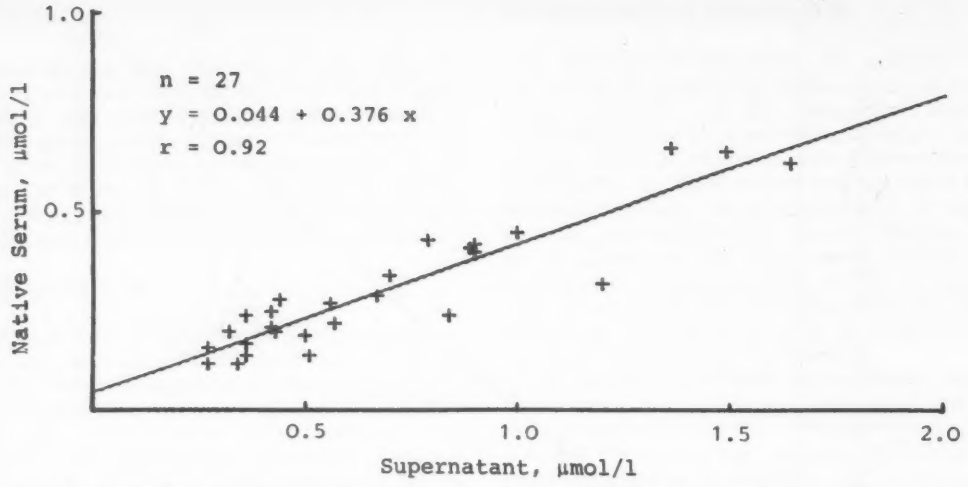

FIGURE 2.—Comparison of results obtained with the enzymatic assay using native serum or supernatant after protein precipitation. Same samples as in fig 1.

dialysis) and supernatant (after dialysis) was the same as prior to dialysis. The mean value of the difference after dialysis is included in figure 3.

Since MTX binds very tightly to DHFR, and this complex can be separated from free MTX by gel chromatography (3), 0.5 ml of a 24-hour patient sample was chromatographed on a Sephadex G-75 column as compared to pure MTX and pure DHFR (fig 4). The patient sample contained 2.2×10^{-6} mol/L of MTX when determined in native serum and 3.26×10^{-6} mol/L of MTX when determined in the supernatant after protein precipitation, ie, 1.06 mol/L of MTX was strongly bound to protein. In each fraction of the eluate, MTX concentrations were determined twice. The closed circles in figure 4C give the concentrations as found in native eluate; the open circles give the concentrations as found in heat-denatured eluate. While in native eluate we only found one main peak of MTX activity in the fractions 19-27, the denaturing of proteins gave rise to some additional MTX activity in the fractions 8-18, ie, in fractions with a retention volume similar to DHFR (fig 4A). The combined fractions 10-18 and 19-27 (18 ml each) were concentrated in an Amicon ultrafiltration cell fitted with a PM 10 membrane to a final volume of 1.5 ml. Figure 4D shows that in the concentrate of fractions 10-18, > 90% of MTX activity was only measurable after protein precipitation, while in the concentrate of fractions 19-27, the majority of MTX activity could be determined directly. The protein that binds MTX (fractions 10-18) eluted with a greater retention volume than the mass of serum proteins (open triangles in fig 4C).

In addition, we injected a rat with 3 mg/kg of MTX iv and killed it 12 hours later, since it is well known that by this time, nearly all of the MTX in the liver is tightly bound to DHFR (4). When using the enzymatic assay, this bound MTX can only be determined after protein precipitation. When 0.5 ml of a 100,000 × *g* supernatant from the rat liver homogenate (containing the MTX-DHFR complex) was chromatographed on the Sephadex G-75 column, the majority of the MTX activity was found in fractions 8-18 and could be determined with the enzymatic assay only after boiling the eluate (results not shown). Thus, during gel chromatography, an MTX-DHFR complex from rat liver behaved exactly like the bound MTX from human serum.

Patients Receiving Intermediate-Dose MTX Therapy

Six adult patients received 14 4-hour infusions of 500-600 mg of MTX as part of their tumor treatment schedule. In these patients on intermediate-dose therapy, 24-hour concentrations in native serum were 2.2×10^{-7} mol/L (range, 1.1-17.5) and had fallen to 3.7×10^{-8} mol/L (range, 1-29) after 48 hours. There was no difference in concentration between native serum and supernatant in the 24-hour values, and the increase in MTX concentration after boiling of the 48-hour values was only 26.1% ± 7.5% ($\bar{x}$ ± SEM).

DISCUSSION

After high-dose MTX therapy, patients have an increased risk of severe toxicity if serum levels are > 10^{-6} mol/L 48 hours after drug infusion, especially if MTX

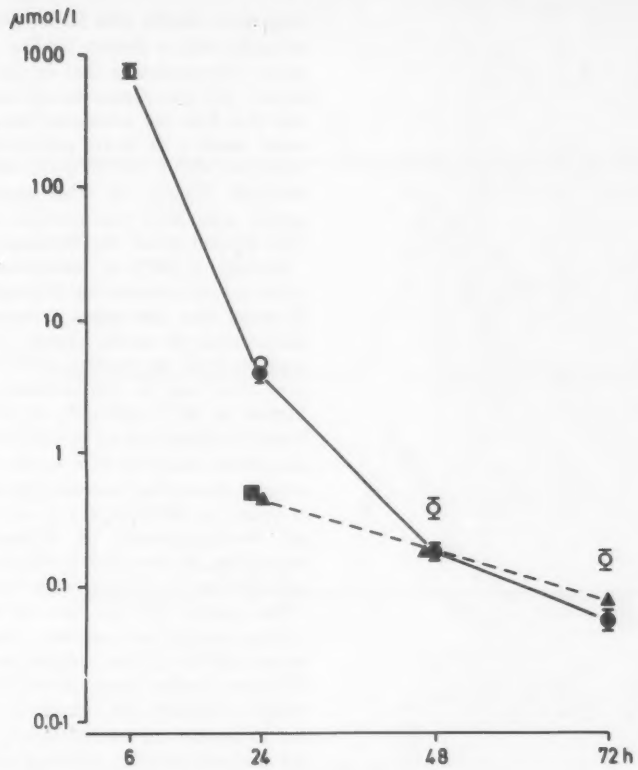

FIGURE 3.—Geometric means and 95% confidence limits of MTX serum concentrations in Patient 1 found during 14 high-dose therapies, where 20 g of MTX was given as a constant infusion over 6 hrs. ● = concentrations as determined in native serum; ○ = concentrations found in supernatant after heat precipitation of proteins; ▲ = mean of the differences between native serum and supernatant; and ■ = mean of the differences between native serum and supernatant after 1 ml of serum was dialyzed for 24 hrs against 1 L of phosphate-buffered saline to reduce free MTX.

elimination continues to be delayed and 72-hour values are $> 10^{-7}$ mol/L [cf, (5)]. These high-risk patients receive additional alkalizing infusions and leucovorin until their serum MTX has fallen below the critical value.

HPLC is a very specific method to determine MTX serum concentrations, and, in addition, offers the possibility of monitoring the concentration of its metabolites concomitantly. However, when using this method for patient surveillance, one has to consider that the accepted limits of MTX serum concentrations for risk of toxicity were obtained using methods without sample cleanup [cf, (5)]. This is remarkable in view of the present finding that in serum from patients on high-dose MTX therapy, protein precipitation leads to the liberation of tightly bound MTX, which is not measured with the enzyme inhibition assay (and probably not with all other assays using native serum). The increase in MTX concentration by boiling was most pronounced in 48- and 72-hour serum samples, ie, those samples mainly used to judge for increased risk of toxicity.

There have been several reports previously comparing HPLC with the more established methods (enzyme inhibition assay, radioimmunoassay). Nelson et al (6) compare HPLC with an enzyme inhibition assay, and conclude that the HPLC method gives lower serum concentrations than the enzyme inhibition assay, since the former is more specific. This conclusion is based on results obtained from one patient and demonstrated in their figure 1. However, their table 1 reveals that four of five patient samples with MTX levels $< 10^{-6}$ mol/L gave considerably higher values with the HPLC method; this agrees with our results. Howell et al (7) also state that their results by HPLC were nearly always lower than those by enzyme inhibition assay; however, they do not mention under what conditions they observed the reverse. Watson et al (8) point out

TABLE 1.—Geometric means of MTX serum concentrations determined enzymatically in native serum (direct) and in the supernatant (super) after protein precipitation*

Patient No. (No. of assays)	6 hrs ($\times 10^{-8}$ mol/L)			24 hrs ($\times 10^{-6}$ mol/L)			48 hrs ($\times 10^{-7}$ mol/L)			72 hrs ($\times 10^{-7}$ mol/L)		
	Direct	Super	Δ	Direct	Super	Δ	Direct	Super	Δ	Direct	Super	Δ
1 (n = 14)												
$\bar{x}$	0.76	0.75	∅	3.3	3.9	0.46	1.83	3.8	1.94	0.57	1.52	0.75
Range	0.51–1.0	0.54–1.0		2.0–5.5	2.5–6.3	0.03–1.06	1.19–2.79	2.66–5.99	1.09–3.81	0.23–0.89	0.94–2.26	0.05–1.72
2 (n = 8)												
$\bar{x}$	1.32	1.39	∅	12.4	15.4	3.3	4.6	10.9	6.2	1.5	4.9	3.3
Range	1.1–1.61	1.2–1.7		8.3–21.0	7.6–29.0	1.0–8.0	2.4–6.6	8.4–16.4	4.8–10.2	0.9–3.3	3.9–8.3	2.2–7.1
3 (n = 6)												
$\bar{x}$	1.12	1.13	∅	13.1	14.9	1.8†	5.1	8.8	2.6	1.6	4.3	2.6
Range	0.81–1.6	0.87–1.43		5.4–43.8	5.4–45.7	0–4.9	1.9–21.3	4.2–28.1	0.7–6.8	0.6–6.7	1.7–14.7	1.0–8.0
4 (n = 4)												
$\bar{x}$	1.3	1.2	∅	3.0	3.8	0.6	1.85	4.8	2.9	0.5	1.85	1.34
Range	1.16–1.51	1.17–1.21		1.4–7.7	1.5–9.8	0.08–2.2	0.86–3.2	2.2–9.3	1.3–6.3	0.27–0.68	1.1–3.0	0.81–2.3

* Δ = the geometric means of the differences.
† Arithmetic mean, since 2 Δ values were 0.

that their results with HPLC are comparable to those obtained with a protein binding assay; however, their table 1 demonstrates that of the 14 pairs of measurements, ten give higher values with the HPLC method and that from the remaining four, three serum samples were taken < 24 hours postinfusion, a period during which we also found no great difference between both methods. Finally, in other reports [eg, (9)], plasma spiked with MTX was used to compare the methods. This will not reveal the difference reported.

Binding of MTX to normal serum proteins can be ruled out as a reason for the observed effect, since (*a*) it would also take place in serum spiked with MTX (calibrators); (*b*) serum protein binding would have to compete with the binding of MTX to the DHFR from beef liver used in the enzymatic assay which is inhibited by MTX with a K_i of 10^{-11} mol; and (*c*) MTX bound to normal serum proteins would leave the Sephadex column with the void volume (fractions 1–10). Procedures denaturing proteins liberate MTX that is tightly bound to DHFR (3,10,11), and, in the present study, gel chromatography on Sephadex G-75 of native patient serum gave strong evidence that the MTX fraction liberated by boiling is also bound to DHFR.

The source and the fate of the circulating MTX-protein complex are unknown. From figure 3, the mean serum half-life of the complex can be estimated to be 20 hours (dashed line). Since DHFR has a molecular weight of 22,500, the complex may be filtered through the glomerule and reabsorbed by the brush border of the proximal tubules. Although this mechanism is only speculative, it could add to the nephrotoxicity of MTX.

MTX binds to DHFR on an equimolar basis (12), and, from animal studies, it was concluded that this is one of the reasons for the persistence of high MTX concentrations in rat liver (1.2 μmol/kg) and kidney over several weeks (13). In the serum of Patient 1, the mean concentration of tightly bound MTX 24 hours after infusion was 0.46 μmol/L, or 1.38 μmol in 3 L of serum (in a patient weighing 70 kg). We believe that this amount is considerable, because it corresponds with that which we have found in 1 kg of rat liver (13).

Patients receiving intermediate-dose MTX therapy only showed a marginal increase in serum MTX concentration after protein precipitation. Therefore, it seems that the amount of tightly bound MTX liberated into serum depends on the MTX dose and may be the result of MTX-induced DHFR synthesis. Actually, Bertino et al (14) found some MTX-induced DHFR synthesis in the leukocytes and erythrocytes of patients receiving MTX therapy.

In conclusion, in the serum of patients receiving high-dose MTX therapy, we found some MTX tightly bound to a protein which could be DHFR. The therapeutic and toxic effects of this complex are unknown. However, if the protein proves to be DHFR, this MTX

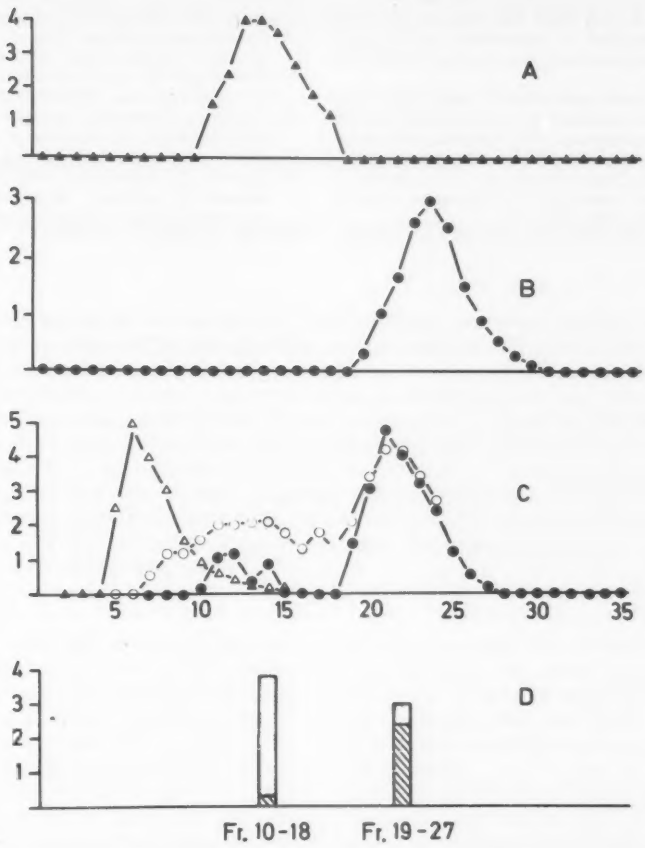

FIGURE 4.—Sephadex G-75 gel chromatography of 0.5 ml of serum from a patient (24-hr sample) on high-dose MTX and MTX (B). ● = MTX concentration as found in native eluate; ○ = MTX concentrations as found in the eluate after heat precipitation of proteins; △ = change in absorbance at 254 nm (arbitrary units) as a measure of protein concentration; and ▲ = DHFR activity. Ordinate: 2-ml fractions. Abscissa: A, mU/ml; B, MTX 10^{-7} mol/L; and C, MTX 10^{-8} mol/L. Combined fractions were concentrated 1:10 by ultrafiltration using an Amicon PM 10 membrane. MTX in the protein-containing concentrate as measured directly ▨ and after protein precipitation □ (D). Abscissa: MTX 10^{-7} mol/L.

must be considered inactive, since MTX already bound to DHFR cannot inactivate additional DHFR. In vitro, MTX is liberated from the complex by protein precipitation. Consequently, any method of therapeutic drug monitoring using protein precipitation for sample cleanup overestimates the concentration of active MTX in patient serum. We believe that the enzyme inhibition assay using the target enzyme, ie, *mammalian* DHFR, and native patient serum is the most appropriate method for MTX drug monitoring. This procedure would measure only MTX (and perhaps metabolites) that can still inactivate the target enzyme.

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Methotrexate: Bioavailability and Pharmacokinetics[1,2]

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Six adult patients with squamous cell carcinoma of the head and neck were treated with single low doses of methotrexate (MTX) (30 mg/m^2) iv, im, and orally in the form of commercial tablets. A randomized crossover design was employed. Plasma concentrations were measured by a modified EMIT assay over a period of 24 hours following each dose. The mean (± SD) parameters following iv MTX were as follows: total-body clearance, 124 (36) ml/minute; V_{ss}, 0.56 (0.18) L/kg; V_λ, 0.69 (0.24) L/kg; and beta-half-life, 3.20 hours. The absolute systemic bioavailability of the oral tablets was 36% (± 10%). After im administration, the systemic bioavailability was 93% (± 14%). Dose-dependent gastrointestinal absorption is suggested as the mechanism for the low availability of the oral tablets. Administration of MTX by the oral route will require further study to determine the optimal method of dosing. [Cancer Treat Rep 69:833–838, 1985]

Methotrexate (MTX), a folic acid antagonist, has been used as a chemotherapeutic agent since its introduction in the late 1940s. Tumors sensitive to MTX include those of the breast, head and neck, and choriocarcinoma. A number of studies have associated the cytotoxic effectiveness of MTX to a tissue-dependent threshold concentration and duration of exposure to the drug (1).

MTX has been administered by the iv, im, and oral routes of administration. Only one study has compared the therapeutic effectiveness of MTX given in the same dose by these three routes (2). The investigators found higher and more sustained MTX levels in those patients who received MTX by the im route.

The iv route is most commonly used to administer MTX, but there may be situations when administration by the im or oral route is necessary. A patient whose veins have been compromised by previous chemotherapy and diagnostic venipunctures may be a candidate for im MTX. The oral route would be an alternative for those patients with minimal muscle mass. Oral administration also offers the possibility for self-administration on an outpatient basis for those patients on maintenance therapy. To provide equivalent therapeutic effectiveness when changing from one route of administration to another, the biologically equivalent dose for each route must be known.

Although MTX has been used in chemotherapy for several years, there has not been a controlled study performed to determine the absolute bioavailability of the oral tablets. There have been several reports of MTX disposition kinetics in the literature; however, the results have been conflicting, partially because of the different analytical methods used to measure MTX in the plasma.

One purpose of this study was to describe the pharmacokinetic parameters obtained from the plasma concentration versus time profile of MTX following iv administration of a conventional dose. The second purpose was to assess the extent to which conventional-dose MTX is absorbed via the im and oral routes so that therapeutically equivalent doses can be recommended.

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MATERIALS AND METHODS

Protocol

Six adults ranging in age from 57 to 76 years with advanced squamous cell carcinoma of the head and neck participated in the study after providing written informed consent. The patients were judged to be in good physical condition despite their disease and had no renal or hepatic dysfunction. Each of the six patients studied received a 30-mg/m^2 dose of MTX by three different routes of administration: iv, im, and oral (in tablet form). MTX tablets of 2.5 mg (Lederle Labs, lot No. 648-570) were dosed to the nearest whole tablet for the oral regimen. MTX sodium (25-mg MTX base/ml; Lederle, lot No. 634-318) for injection was used for the iv and im doses. Patients were sequentially entered into regimens which had been randomized by factorial design so that no two patients completed the three portions of the study in the same sequence. All patients received their MTX doses 1 week apart for 3 consecutive weeks.

The iv dose was administered by an iv push over 1 minute followed by 10 ml of normal saline. The im dose was administered into the gluteal muscle. The oral tablets were ingested whole at one time with 8 oz of water. Each patient was required to fast for 8 hours before and 4 hours after administration of each dose by the iv, im, and oral routes. Blood samples were collected through an indwelling venous cannula for 24 hours following dosing. The plasma fraction was separated and kept frozen until assayed.

Analytical Methods

Plasma samples were assayed for MTX concentration by the homogenous enzyme immunoassay technique as modified by Finley et al (3) using a centrifugal analyzer (COBAS BIO, serial No. 18-2028, Roche Analytical, Nutley, NJ). The commercially available assay kit (EMIT, Syva Co, Palo Alto, CA) was used in these analyses. Patient samples that yielded concentrations above the range of the standard curve (0-675 ng/ml) were diluted with a 5% albumin solution and reanalyzed. The lowest MTX concentration that could be measured accurately and precisely was 45 ng/ml.

Data Analysis

The data obtained from the plasma concentration (C) versus time (t) profile of MTX following iv, im, and oral doses were analyzed by a "model-independent" method. This method requires that the data must be from a mammillary system describable by first-order processes with elimination from a central compartment (4).

Initially, the iv MTX data in the postdistribution phase of the plasma concentration-time plot were fit, using linear regression, to the equation

$$\ln C = \ln C^0 - \lambda_n t \qquad [1]$$

where C^0 is the extrapolated zero-time concentration expressed as ng/ml and λ_n is the terminal disposition rate constant in hr^{-1}. The half-life ($t_{1/2}$) was determined using the relationship

$$t_{1/2} = \frac{0.693}{\lambda_n} . \qquad [2]$$

To obtain total-body clearance (Cl_s) for MTX, it was necessary to obtain the total area under the plasma concentration versus time curve from time zero to infinity (AUC). The area from time zero to the first concentration-time point in the postdistribution phase (AUC_0^t) was calculated using the trapezoidal rule. The area under the curve from the first concentration-time point to the postdistributive phase (C_t) to time infinity (AUC_t^∞) was calculated from the equation

$$AUC_t^\infty = \frac{Ct}{\lambda_n} . \qquad [3]$$

The concentration used for C_t was the theoretical value of C+ obtained from the linear regression equation, not the observed concentration. AUC was then obtained using the equation

$$AUC = AUC_0^t + AUC_t^\infty . \qquad [4]$$

With the calculation of AUC, the volume of distribution (V_d) and Cl_s were obtained from the following equations:

$$Cl_s = Dose/AUC \qquad [5]$$

and

$$V_d = Cl_s/\lambda_n . \qquad [6]$$

The volume of distribution at steady-state (V_{ss}) was calculated using the equation

$$V_{ss} = Dose \frac{AUMC}{AUC^2} \qquad [7]$$

where AUMC is the total area under the curve of the product of time and plasma concentration versus time from time zero to infinity (area under the first moment of the plasma curve). The area from zero to the first time-point in the postdistributive phase was again calculated using the trapezoidal rule.

The total area under the plasma concentration versus time curve after im and oral administration of MTX was obtained in the same manner as for the iv data. The data in the postabsorptive, postdistribution portion of the plasma concentration-time curve were fit using linear regression from which the parameters λ_n and $t_{1/2}$ were ob-

tained. Area under the curve calculations for quantitating the extent of absorption were accomplished by determining the area from time zero to the first concentration-time point in the postabsorptive, postdistributive phase using the trapezoidal rule, and adding the area from this concentration-time point to time infinity calculated from equation [3].

The absolute intramuscular and oral bioavailability of MTX (F) was calculated by the equation

$$F = \frac{(AUC)_{IM \text{ or } PO}}{(AUC)_{IV}} \times \frac{X^{\circ}_{IV \text{ or } PO}}{X^{\circ}_{IM}} \quad [8]$$

where X^0 is the dose of MTX administered, and the subscripts refer to the iv, im, and oral values.

The elimination half-lives and areas under the plasma concentration versus time curve after the iv, im, and oral administration of MTX were analyzed by analysis of variance, repeated measures design (5). Each of the analyses of variance was performed by the Statistical Package for the Social Sciences (SPSS), RELIABILITY subprogram (6). The maximum plasma concentration and the time to maximum plasma concentration after the im and oral administration of MTX were analyzed by the paired t-test method (7).

RESULTS

Figure 1 displays the plasma MTX concentration versus time curves, including the line of best fit derived from a linear regression of points in the postabsorptive, postdistributive phase for each patient after iv, im, and oral dosing. The pharmacokinetic disposition parameters after iv administration of a 30-mg/m^2 dose are summarized in table 1.

The data for Patient 4 from the iv course of therapy were excluded from statistical analysis. Although the half-lives after im and oral administration were in close agreement, the iv value was approximately threefold greater. Also, the half-life obtained for this patient following iv administration differed significantly from the iv values obtained for the other patients. This larger value was a result of both a larger volume of distribution and a smaller clearance relative to the other patients. To our knowledge, this patient was not taking any drugs that would inhibit the elimination or increase the intravascular distribution of MTX. His renal function was also within the range of the other patients. Plasma samples from the iv course of therapy were reanalyzed and the plasma concentration values which resulted were equiva-

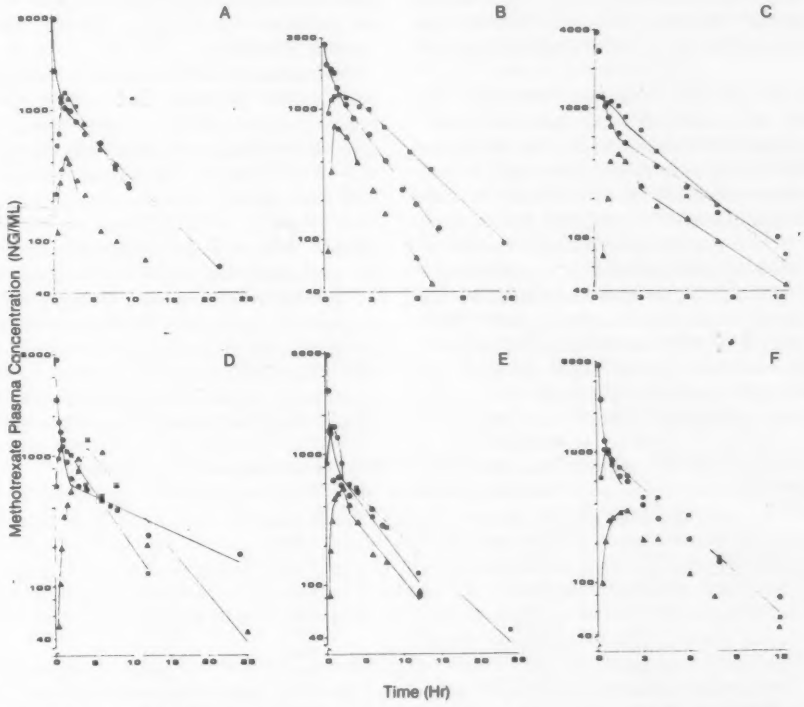

FIGURE 1.—A panel of plasma disappearance-time plots for MTX given to 6 cancer patients (A–F, corresponding to Patients 1–6, respectively). Each plot shows MTX disappearance following 3 different routes of drug administration: ● = iv; ■ = im; and ▲ = oral.

TABLE 1.—Pharmacokinetic parameters of MTX following iv administration

Patient No.	Cl_s (ml/min)	V_{ss} (L/kg)	V_d (L/kg)	λ_n	$t_{1/2}$ (hrs)
1	75	0.60	0.68	0.141	4.93
2	114	0.28	0.32	0.375	1.85
3	133	0.77	0.99	0.179	3.92
4	58*	0.88*	1.05*	0.610*	11.36*
5	123	0.56	0.72	0.18	3.76
6	175	0.58	0.76	0.205	3.37
Mean	124	0.56	0.69	0.217	3.20†
± SD	± 36	± 0.18	± 0.24		

* Not used in the calculation of the mean and SD.
†Harmonic mean.

lent to those obtained after the first analysis.

The overall mean elimination half-life for MTX for the remaining five patients following iv administration was 3.20 hours (range, 1.85–4.93). The mean and range for clearance, and the volumes of distribution V_{ss} and V_d were 124 ml/minute (75–175), 0.56 L/kg (0.28–0.77), and 0.69 L/kg (0.32–0.99), respectively.

Table 2 lists the individual half-lives of MTX for each route of administration. The mean elimination half-life for MTX after im administration was 3.28 hours (range, 2.65–5.27). After oral administration, the mean elimination half-life was 3.21 hours (range, 1.58–4.29). No statistically significant difference was found between the mean elimination half-lives after iv, im, and oral administration.

Absorption of MTX from both the muscle and the gastrointestinal tract appeared to be rapid, as measurable concentrations of MTX were detected in the plasma 15 minutes after the administration of the dose in most cases. Measurable concentrations of MTX were not observed until 30 minutes after an oral dose in Patients 1 and 3. The mean time to reach maximum plasma concentration (t_{max}) after im administration was 0.85 ± 0.49 hours (range, 0.25–1.5). After oral administration, the mean time to attain maximum concentration was 1.55 ± 0.51 hours (range, 0.75–2.0). No statistically significant difference was found between the mean values of t_{max} after im and oral administration. The mean absolute im bioavailability was 0.93 ± 0.14 (range, 0.78–1.11).

The mean absolute oral bioavailability was found to be 0.36 ± 0.10, with a range of 0.24–0.52. Individual patients' oral bioavailability values are listed in table 3.

DISCUSSION

A number of previous studies have described the pharmacokinetic disposition of MTX. Henderson et al (8) described a mean MTX elimination half-life of 2.2 hours in ten patients following a 0.1-mg/kg dose of tritiated MTX administered either orally or iv. In 26 patients with psoriasis, Halprin et al (9) reported that the MTX half-life ranged from 2 to 10 hours following single oral or im doses of 2.5–25 mg.

Huffman et al (10) have described triphasic MTX pharmacokinetics following the iv administration of 30 mg/m^2 of tritiated MTX to 22 cancer patients. The initial distribution phase in this study was rapid, with a half-life of 0.45 ± 0.11 hours. The second phase had a half-life of 3.49 hours. A third phase of elimination was reported to have a half-life of 26.99 hours. It was postulated that enterohepatic recycling of drug might be responsible for this prolonged third phase. The plasma was analyzed for MTX for up to 96 hours after dosing by determining total radioactivity (including MTX metabolites and tritium breakdown products) via liquid scintillation spectrometry (10). This MTX analytic method could spuriously lengthen the apparent terminal MTX half-life, produce a greater AUC, and, hence, a lower clearance.

TABLE 2.—Comparison of the elimination $t_{1/2}$ of MTX following iv, im, and oral administration

	Iv		Im		Oral	
Patient No.	λ_n (hr^{-1})	$t_{1/2}$ (hr)	λ_n (hr^{-1})	$t_{1/2}$ (hr)	λ_n (hr^{-1})	$t_{1/2}$ (hr)
1	0.1406	4.93	0.2016	3.44	0.1617	4.29
2	0.3754	1.85	0.2611	2.65	0.4373	1.58
3	0.1768	3.92	0.2315	2.99	0.1749	3.96
4	0.0610*	11.36*	0.1984	3.49	0.1638	4.23
5	0.1841	3.76	0.1316	5.27	0.1695	4.09
6	0.2054	3.37	0.2455	2.82	0.1884	3.68
Mean	0.2165	3.20†	0.2116	3.28†	0.2159	3.21†

* Not used in the calculation of the mean and SD.
†Harmonic mean.

TABLE 3.—Absolute oral bioavailability of MTX

Patient No.	AUC_0^{∞} (oral) (ng·hr/ml)	AUC_0^{∞} (iv) (ng·hr/ml)	Dose (mg) Iv	Dose (mg) Oral	F
1	2396	9948	45	45	0.24
2	1916	5865	40	40	0.33
3	2193	5645	45	45	0.39
4	9719	13440	47	47.5	0.72*
5	3545	6763	50	50	0.52
6	1665	5250	55	55	0.32
Mean					0.36
± SD					± 0.10

* Not used in the calculation of the mean and SD.

Nevertheless, the results of the current study are in general agreement with most of these previously reported values. Our average MTX half-life of 3.20 hours is similar to the half-life for the second phase of elimination observed by Huffman et al (10). It is also within the range of half-life values reported by Halprin et al (9).

The mean plasma clearance of MTX in this study was found to be 124 ± 36 ml/minute. This value is higher than those reported after similar iv doses by Lawrence et al (11) (62 ml/minute) and Huffman et al (10) (31 ml/minute). However, these earlier studies described values of renal clearance, not total plasma clearance. This may explain why our value is higher, since renal clearance accounts for only 80%–90% of the total plasma clearance of MTX. The shorter length of sampling time in the current study also may have caused an underestimation of the plasma clearance.

In this study, the mean volume of distribution of steady-state (V_{ss}) ranged from 15.9 to 38.7 L, with a mean of 29.9 L. Huffman et al (10) reported V_{ss} to be 73.3 ± 15.6 L following the iv administration of 30 mg/m^2 of MTX to 22 patients. This difference may again be that total radioactivity was measured and the fact that the body weights of the subjects in Huffman's investigation were higher than those in our study.

Only two studies have investigated the pharmacokinetics of MTX following im administration (2,9). Both suggested that the absorption of MTX was rapid and complete. However, in neither study was the drug administered iv, so the absolute availability of oral doses remained unknown. Following single im doses of 25–50 mg, Halprin et al (9) found that peak levels of MTX were achieved at 2 hours following the dose. Freeman-Narrod et al (2) also reported that the peak level occurred 2 hours following a 15-mg/m^2 dose. This concurs with the current results, and it appears that virtually all of the im dose of MTX administered is available to the systemic circulation (table 3).

Based on a comparison of the AUCs from the oral and iv experiments, the mean absolute systemic availability of the tablets is 36%. This value is 25% lower than the value (47.7%) reported by Wan et al (12), but is similar to the recent findings of Pinkerton et al (13), where the mean bioavailability was 42% but ranged from 6% to 91%. Balis et al (14) have also observed significant interpatient bioavailability differences which appear to be dose-dependent, ranging from 23% to 95% (mean, 63%). Likewise, our results suggest that approximately 60% of a 30-mg/m^2 dose is not available to the systemic circulation. This poor availability would not appear to be a result of first-pass metabolism, since the nonrenal clearance of MTX is only a small fraction of liver blood flow. However, Wan et al (12) found that metabolites of MTX in the urine constituted 35% of a 30-mg/m^2 oral dose, compared to 6% for the same dose administered iv. These findings suggest that MTX metabolism by intestinal bacteria in the gastrointestinal tract occurs and/or that there is metabolism by intestinal tissue.

Dose-dependent absorption of MTX from the gastrointestinal tract may also contribute to the low availability found after oral administration in this study. Chungi et al (15) have shown that the absorption of MTX from the lumen of the rat small intestine, in situ, is saturable at relatively low drug concentrations and obeys Michaelis-Menten kinetics. Wan et al (12) administered MTX orally to humans at two dosage levels (30 and 80 mg/m^2) and found that absorption was significantly lower at the higher level.

Steele et al (16) administered an oral syrup of MTX by two regimens: (*a*) a single 100-mg dose; and (*b*) 25 mg every 2 hours for four doses to eight cancer patients. The elimination half-life of MTX was found to be the same after each regimen. Area under the plasma concentration versus time curve was significantly greater following the subdivided dose by a ratio of almost 2:1. Stuart et al (17) also studied the absolute oral bioavailability of a 50-mg/m^2 dose of MTX syrup in six adults who were treated for a solid tumor. They found the average availability to be 26%. One would expect the absorption to be greater with a syrup than with the tablets; however, their results support our observation of low oral availability and are consistent with the theory of a dose-dependent absorption of MTX from the gastrointestinal tract.

A problem with the oral route of MTX administration is that the drug is only available in 2.5-mg tablets. Thus, our patients were required to take from 16 to 22 tablets

for a 30-mg/m^2 dose. This poses a substantial problem for patients who have difficulty swallowing. The formulation of a tablet of higher strength or the pharmaceutical compounding of MTX into a more absorbable liquid dosage form would be alternative solutions to this problem. Based on the reduced absolute oral availability and the unpredictable potential for saturable intestinal absorption seen in our study, the oral route of administration cannot be routinely recommended for MTX.

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Clinical Pharmacokinetics of Vindesine Infusion[1,2]

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Fifteen patients were given vindesine (VDS) either by iv bolus injections at doses ranging from 0.7 to 1.2 mg/m² or by 5-day infusions (total dose: 5 mg/m²), in combination with cisplatin (20 mg/m²/day) and bleomycin (6 mg/m²/day) for 5 days. For bolus injections, the total dose of VDS in one treatment was completed to 5 mg/m² by infusion on Days 4 and 5. Drug concentrations in plasma and urine were measured by radioimmunoassay. Plasma concentration decay curves after bolus injection presented the expected triphasic shape, whereas for infusions, plasma concentrations increased and reached steady-state after about 30 hours and declined in a biphasic way after infusion discontinuation. Steady-state concentrations ranged from 4 to 15 μg/L and showed important variations among patients. Clearances estimated from the area under the concentration-time curves were significantly smaller for infusions than for bolus injections. This observation was interpreted as an indication of VDS pharmacokinetic nonlinearity. Terminal half-lives and renal clearances were not significantly different for the two types of administration. Toxicity of the treatment was generally limited, except for one major renal failure; two patients showed objective tumor regression after therapy. [Cancer Treat Rep 69:839–844, 1985]

Vindesine (VDS) is a semisynthetic vinca alkaloid derived from vinblastine (1,2). It has been investigated in numerous clinical trials against a wide variety of tumors, both as a single agent and in combination therapy. Its clinical pharmacokinetics have been studied in cancer patients after rapid iv injections. It is now well-documented that plasma concentration decay curves are triphasic, and this has been interpreted in terms of a three-compartment open mammillary model (3,4). Important quantitative differences have been observed between VDS and other vinca alkaloids such as vinblastine and vincristine with respect to clearances, apparent half-lives of elimination, toxicity, and maximal tolerated doses (5,6). Large variations among patients are typical of vinca alkaloid pharmacokinetics (5,6).

Several clinical trials suggested that long-term infusions may be more efficient than iv boluses (2,7,8). Sustained exposure to relatively high concentrations of these phase-specific agents might explain these results. However, this remains conjectural and data published by Jackson et al (9) for vincristine, by Lu et al (10) for vinblastine, and recently by Jackson et al (11) for VDS indicated that vinca alkaloid clearances might differ between bolus injection and slow infusion. Our own preliminary investigations (12) and the dose-dependence of VDS pharmacokinetics we recently observed (13) prompted us to compare plasma concentrations and urinary excretion of VDS given at random as bolus injections or infusions to a group of 15 patients who were part of a clinical trial of a combination of VDS, cisplatin, and bleomycin (14). Results of this kinetic study are reported in this paper together with a brief summary of the clinical incidence (short-term responses and toxicity) of the treatment.

MATERIALS AND METHODS

Patient Eligibility

Fifteen patients with histologically documented neoplastic diseases (table 1) were entered in this study. Their eligibility was based on the following criteria: wbc count $> 3000/mm^3$; platelet count $> 100,000/mm^3$; serum creatinine < 120 μmols/L; and no clinical or biochemical abnormalities of hepatic or renal function.

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TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Sex	Primary tumor site	Body area (m^2)	Protocol*	Total dose (mg)/course			Prior treatment
						Cisplatin	Bleomycin	VDS	
1	44	M	Tongue, esophagus	1.8	A, B, C	180	50	9	None
2	62	M	Lung	1.8	A, B	180	55	9	None
3	53	M	Larynx	1.8	B, A	175	50	9	Chemotherapy; radiotherapy
4	43	M	Lung	1.6	B, A	165	50	8.5	Surgery
5	56	M	Lung	1.7	A	175	50	8	None
6	74	M	Larynx	1.6	A	160	50	9	None
7	57	M	Lung	1.8	A	180	50	9	None
8	56	M	Larynx	1.5	A	150	45	8	None
9	72	F	Lung	1.5	A	150	0	7.5	None
10	63	M	Tongue	1.5	A	150	0	8	None
11	64	M	Hypopharynx	1.85	A	150	50	8	None
12	59	M	Hypopharynx	1.7	A	150	50	8	None
13	58	M	Lung	2.0	B	200	60	10	None
14	51	M	Lung	1.8	B	180	55	9	None
15	58	M	Lung	1.8	B	190	50	9	None

* Details about Protocols A (infusion) and B and C (boluses) are given in table 2. Letters refer to sequence of treatments when successive administrations were given. Delay between treatments was 1 mo.

Treatment

The treatment was a combination of VDS, cisplatin, and bleomycin. Detailed protocols are described in table 2. VDS (Eldisine; Eli Lilly and Co) was given as a 5-day infusion via a central venous catheter. The daily dose was mixed in 500 ml of 5% dextrose. Constant-infusion pumps were not used because patient consent was generally not obtained. When given as a bolus, VDS was diluted in 5 ml of 5% dextrose and injected through the catheter in < 2 minutes. Cisplatin was diluted in 125 ml of 5% dextrose and administered as a 5-day infusion. Bleomycin was injected after dilution in 5 ml of 5% dextrose as a bolus. Hydration was performed during the 5 days of infusion with 1 L of 10% mannitol and 500 ml of 5% dextrose.

Sample Analysis

For iv boluses, blood samples were collected a few minutes before injection, then 5, 10, 15, and 30 minutes and 1, 2, 3, 4, 6, 12, 24, 36, 48, 60, and 72 hours after injection. For infusions, blood samples were collected before administration and after 1, 2, 6, 12, 24, 36, 48, 60, 72, and 96 hours of infusion. After infusion was discontinued, blood samples were again collected after 5 min-

TABLE 2.—Chemotherapy regimens*

Protocol	Drug	Dose	Mode
A	VDS	5 mg/m^2	5-day infusion
	Cisplatin	20 mg/m^2/day	5-day infusion
	Bleomycin	6 mg/m^2/day	Bolus
B	VDS	2 mg	Bolus on Day 1; no VDS on Days 2 and 3
		3 mg/day	Infusion starting on Day 4†
	Cisplatin	20 mg/m^2/day	5-day infusion
	Bleomycin	6 mg/m^2/day	Bolus
C	VDS	4 mg	Bolus on Day 1; no VDS on Days 2 and 3
		2 mg/m^2/day	Infusion starting on Day 4†
	Cisplatin	20 mg/m^2/day	5-day infusion
	Bleomycin	6 mg/m^2/day	Bolus

* Protocols A, B, and C were randomized. Delay between 2 successive courses was 1 mo.
†VDS infusion was continued on Days 4 and 5 to a total dose of 5 mg/m^2 (bolus + infusion).

utes and 1, 3, 6, 12, and 24 hours. Blood collection was from an arm vein and heparinized tubes were used. Tubes were kept at 4°C until centrifugation (10 minutes at 1000 × *g*). Plasma was stored at -20°C until analyzed. Each urine output was collected separately over 72 hours for bolus injections and over 120 hours for infusions; volume was measured and an aliquot (5 ml) was kept frozen until analysis.

VDS concentration in plasma and urine was measured by radioimmunoassay according to the technique described by Rahmani et al (15), using [^{125}I]vinblastine-glycyl-tyrosine and rabbit antivinblastine antiserum (Lilly Research Laboratories). Concentrations were calculated by interpolation on a standard reference curve. The sensitivity of the assay was better than 0.1 μg/L (1.2×10^{-10} M) and the reproducibility was around 9% (15).

Pharmacokinetic Analysis

Apparent half-lives of elimination were calculated by least-square regression on terminal data points. Area under concentration-time curves (AUC) were calculated according to the trapezoidal rule (16) and extrapolated to infinite time using the experimental half-lives or a default value of 24 hours (3,4,13) when the calculation was not possible.

Cumulated urinary excretion was calculated by adding up the amount of VDS excreted within 72 hours for bolus injections or 120 hours for infusions. Renal clearances were calculated by dividing the total amount of VDS excreted in the urine by the plasma AUC (16).

RESULTS

Systemic Clearance and Apparent Elimination Half-Life

Figure 1 shows the plasma concentration kinetics from studies on Patient 1, who was first treated with 9 mg of VDS by infusion, then by two successive iv boluses of 2 and 4 mg of the drug. The interval between successive examinations in the same patient was about 1 month; no detectable amount of VDS remained in plasma or urine at the time of the next administration. Such curves are rep-

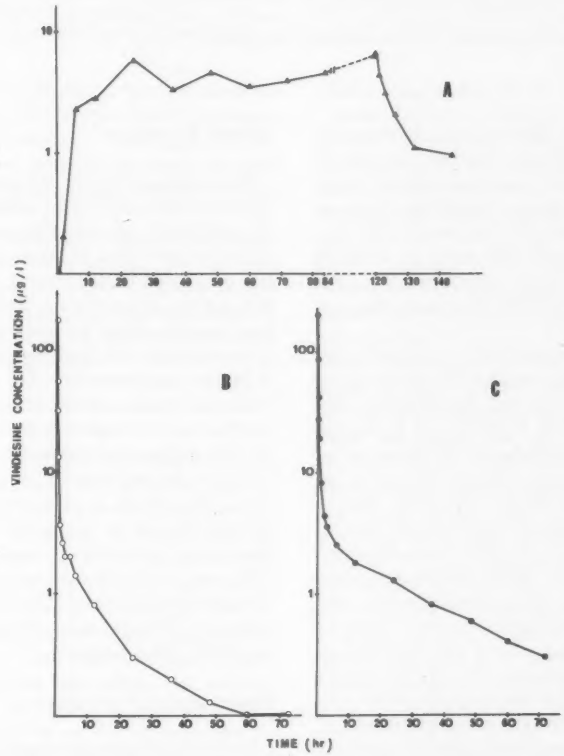

FIGURE 1.—Patient 1 was given 9 mg of VDS by 5-day continuous infusion (panel A), then 2 mg (panel B) and 4 mg (panel C) of VDS at 1-mo intervals as bolus injections.

TABLE 3.—VDS pharmacokinetic parameters

Patient No.	Protocol*	VDS dose (mg)	AUC (hr·μg/L)	Systemic clearance (L/hr/m²)	Terminal half-life (hrs)†,‡	Urinary excretion‡,§ (% of dose)	Renal clearance (L/hr/m²)‡
1	A	9	448	11.1	20.3 ± 8.4	4.4	0.49
2	A	9	539	9.3	17.2 ± 8.6	4.6	0.42
3	A	9	459	10.9	ND	ND	ND
4	A	8.5	746	7.1	19.4 ± 6.5	ND	ND
5	A	8	437	10.8	17.7 ± 0.7	5.2	0.56
6	A	9	1475	3.8	48.2 ± 16.1	6.9	0.26
7	A	9	783	6.4	ND	4.3	0.28
8	A	8	447	11.9	ND	ND	ND
9	A	7.5	431	11.6	ND	8.4	0.97
10	A	8	406	13.1	17.7 ± 0.7	5.8	0.85
11	A	8	930	4.6	11.6 ± 1.4	ND	ND
12	A	8	588	8.0	10.3 ± 3.5	ND	ND
1	B	2	64.7	15.4	20.4 ± 3.6	3.0	0.52
2	B	2	50.8	18.9	16.8 ± 2.8	5.2	1.14
3	B	2	73.0	13.7	35.3 ± 2.1	ND	ND
4	B	2	48.0	22.3	16.5 ± 1.7	4.6	1.20
13	B	2	117.6	8.0	30.3 ± 2.3	ND	ND
14	B	2	47.2	20.1	24.9 ± 0.8	ND	ND
15	B	2	147.1	7.1	14.5 ± 2.1	ND	ND
1	C	4	117.6	17.6	22.8 ± 0.6	3.1	0.58

*See table 2 for details.
†Mean ± SD.
‡ND = not determined.
§Cumulated over 120 hrs (infusions, Protocol A) or 72 hrs (boluses, Protocols B and C).

resentative of VDS kinetics. After iv bolus, plasma concentration decays were triphasic. However, because of the expected nonlinearity (13), these data were not fitted to a linear compartmental model. Clearances were assessed from AUC and normalized to body area. Half-lives were calculated by least-square regression on terminal data points (12–72 hours). Large variations among patients typical of VDS pharmacokinetics were observed for these parameters (table 3).

During VDS infusions, plasma levels plateaued after about 30 hours, with some fluctuations that were not entirely accounted for by infusion rate instabilities; they then decreased in an apparently biphasic way at the end of the infusion. Terminal postinfusion half-lives, tentatively calculated from samples collected from 6 to 24 hours after infusion discontinuation, were very close to those measured after iv bolus (Patients 1, 2, and 4). Again, large variations among patients were observed, with respect to both terminal half-lives and clearances (table 3). The most striking observation, however, was that systemic clearances were smaller for the infusions. This was true when the same patient received VDS as both iv bolus and infusion (Patients 1–4, table 3), and did not depend on which was given first (table 1). When analyzed using the Wilcoxon test (17), the distributions of clearances for all patients considered together appeared to be significantly different for the two modes of administration ($P < 0.01$).

Urinary Excretion

An example of cumulative urinary excretion plots after bolus injection and during infusion is shown in figure 2. We previously reported that excretion rates and plasma concentration after iv bolus were correlated (13). This was confirmed in the present study for bolus injections (not shown). For continuous infusions no such correlation was possible, partly because of fluctuating plasma levels at steady-state and partly because of the narrower range of plasma concentrations. The excretion rates, however, remained fairly constant from the onset of the plasma steady-state concentration to the end of the infusion (fig 2). The urinary excretion of VDS, cumulated over 72 hours for boluses and 120 hours for infusions, was low (3%–8.4% of dose, table 3); level of excretion apparently did not depend on the mode of administration. Renal clearances, calculated as the ratio of cumulated urine VDS over AUC (16) and normalized to body area, were found between 0.26 and 0.97 L/hour/m^2. This was not significantly different from clearances observed after iv bolus (0.52–1.20 L/hour/m^2).

Responses and Toxicity

Patient characteristics regarding tumor types and previous therapy are shown in table 1. One patient with larynx carcinoma (Patient 3) and one with tongue carci-

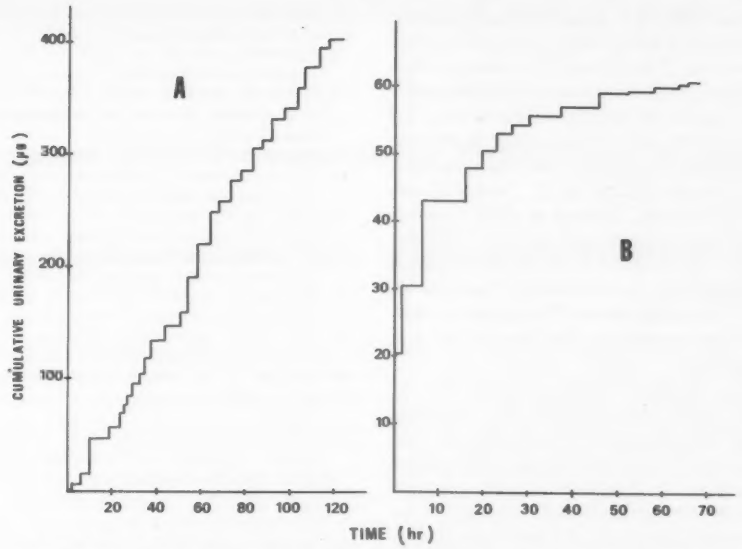

FIGURE 2.—VDS concentrations in urine were measured by radioimmunoassay, and cumulative urinary excretion of VDS (expressed as % of dose) was plotted as a function of time for continuous infusion (total dose, 9 mg; Patient 1, panel A) and after bolus injection (dose, 2 mg; Patient 1, panel B).

noma (Patient 10) showed significant tumor regression after one cycle of combination therapy. Partial responses were also observed for Patients 1, 7, 11, and 12. The other patients had stable or progressive disease or could not be evaluated because of infection (Patients 8, 9, and 13).

Toxic effects with both treatment regimens were similar to previously published observations. We observed one patient with major renal failure after the second course (Patient 3), one with myelosuppression (Patient 7), one with paralytic ileus (Patient 10), and three with serious infections (Patients 8, 9, and 13).

DISCUSSION

Continuous infusions of VDS over relatively long periods of time have now been introduced into many experimental chemotherapy regimens. Potential advantages of vinca alkaloid long-term infusions, such as increased antitumor activity (2,7) with little or no increase in toxicity (18), have been reported. For example, high remission rates resulted from long-term infusion of VDS or vinblastine in patients with hematologic malignancies (19), breast cancer (20), or head and neck cancers (21), even when iv bolus injections had failed. The present study was not designed to evaluate the influence of the mode of VDS administration on antitumor responses or toxicity. The association of VDS, bleomycin, and cisplatin was reasonably well-tolerated and gave an interesting number of responses for such refractory diseases. Its clinical value will be discussed separately.[6]

Most pharmacokinetic data available so far about VDS concern iv bolus injections (3,4). Continuous infusion of VDS was shown to maintain relatively high plasma concentrations (11). This was confirmed in this study, where VDS plasma concentrations ranged between 4 and 15 μg/L (5×10^{-9} to 2×10^{-8} M), levels which have been shown cytotoxic to mammalian cells in culture (22). By contrast, plasma concentrations fall to < 1 μg/L in < 48 hours after iv bolus injection.

Normalized to patient body area, clearances after iv bolus injections were around 15.4 L/hour/m^2 (SD, 5.5), similar to that reported earlier (13). However, systemic clearances were found to be significantly ($P < 0.01$) lower when the drug was given by infusion (mean, 9.1 L/hour/m^2; SD, 3.0). Half-lives at the apparent elimination phase did not show any significant difference between infusion (postinfusion half-life) and bolus. This parameter has consistently been found to distribute around a mean value of 24 hours (3,4,11,13). Renal clearance was not significantly different for the two modes of administration, but slightly increased renal clearance for bolus as compared to infusion is not ruled out. For bolus injections, renal clearances have the same range as that reported earlier for VDS single-agent therapy (13). Thus, combined treatment with bleomycin and cisplatin did not appear to appreciably alter VDS pharmacokinetics.

In a preliminary study of 14 patients with hematologic

[6]Kleisbauer JP, et al. Manuscript submitted for publication.

malignancies given VDS as a single chemotherapeutic agent (12), either by 1.5-5-day infusions (2.3-3 mg/m^2) or by bolus injections (1.5-2.3 mg/m^2), average clearances, evaluated from AUC, were lower for infusions (6.7 ± 3.3 L/hour/m^2) than after bolus injections (13.8 ± 7.7 L/hour/m^2). This previous study did not allow us to reach a statistically significant conclusion, but the agreement with the present study is striking. In the absence of concomitant antitumor therapy, Jackson et al (9) observed similar effects for vincristine (9) and recently VDS infusions (11), and data published by Lu et al (10) suggested that vinblastine might give the same result. Thus, the observed influence of dose schedule on VDS systemic clearance is probably not a result of the association with cisplatin and bleomycin.

The dependence of systemic clearance on the protocol of administration could be explained by nonlinear pharmacokinetics; this has already been strongly suggested by the apparent dose-dependence of VDS kinetics after iv bolus injection (15). Nonlinear kinetics are possibly a general feature for vinca alkaloids. It could be argued that this is merely the result of a lack of specificity of the radioimmunoassay used in every study. This cannot be answered, since no definitive study of vinca alkaloid metabolism has yet been published. However, the limited data available so far indicate that the proportion of parent compound found in plasma after iv injection must be high (23). In addition, the radioimmunoassay is reasonably specific (15) and putative cross-reactive metabolites must present only minor structural changes with respect to the parent compounds. It would then be very useful to determine the toxicity and the possible antitumor activity of such metabolites. On the other hand, saturation of some binding or transport mechanisms could induce increased elimination at higher doses. Thus, the mechanisms of vinca alkaloid distribution, metabolism, and elimination have to be worked out before a model accounting for vinca alkaloid nonlinear pharmacokinetics might be proposed. Such a model will eventually provide a means to calculate optimal doses and to improve the safety and the efficacy of the treatments (24).

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Selective Cytotoxicity of 5-Hydroxyuridine for Human Colon Adenocarcinoma Cells[1,2]

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The cytotoxicity of 5-hydroxyuridine (OHUrd), 5-FU, and 5-fluorodeoxyuridine was determined by colony assays for three human colon adenocarcinoma cell lines and for a cell line derived from normal fetal intestinal cells. All three tumor cell lines were more sensitive to OHUrd than were the FeInt cells, whereas 5-FU was more toxic to the latter. 5-Fluorodeoxyuridine was substantially more cytotoxic to only one of the tumor cell lines compared to the normal cells. In HT-29 tumor cells, OHUrd cytotoxicity could be prevented by coincubation with uridine or cytidine but not by pyrimidine deoxyribonucleosides or by purine nucleosides. Compared to OHUrd, 5-hydroxyuracil was much less cytotoxic for HT-29 cells. The increased cytotoxicity of OHUrd for these tumor cells compared to fetal intestinal cells may implicate biochemical differences that might be exploitable for improved chemotherapy. Direct comparison of drug sensitivity of colon tumor cells compared to normal counterpart cells may provide a method of screening agents selective for these tumor cells. [Cancer Treat Rep 69:845–850, 1985]

Colorectal cancer responds poorly to all known chemotherapeutic agents. The average response rate for the widely used drug 5-fluorouracil (FUra), administered systemically, is about 20% for patients with inoperable disease (1,2). Cytotoxicity for normal proliferating cells of the intestinal mucosa is a major dose-limiting toxic effect of FUra, 5-fluorodeoxyuridine (FdUrd), and several other anticancer drugs. Agents which are selectively toxic for malignant colon cells compared to normal mucosal cells might be more effective drugs for this disease. We have therefore initiated a program of screening potential agents for their relative cytotoxicity for human colon tumor cells compared to normal fetal intestinal (FeInt) cells.

One compound tested was 5-hydroxyuridine (OHUrd), first synthesized by Levene and La Forge (3), which has been found to inhibit growth of bacteria (4), viruses (5), and murine ascites tumors (6). Intracellularly, OHUrd is phosphorylated to its mono-, di-, and tri-phosphate derivatives. The monophosphate nucleotide is a potent inhibitor of orotidylate decarboxylase from Ehrlich ascites tumor cells (7), and the triphosphate nucleotide inhibits DNA-dependent RNA polymerase and can also be incorporated inefficiently into RNA (8).

Very little is known about the effects of OHUrd on human cells. We report here on the cytotoxic effects of OHUrd on three lines of human colon tumor cells and on a cell line derived from normal fetal intestine, and we also report on some initial biochemical studies on the effects of OHUrd and of 5-hydroxyuracil (OHUra) on tumor cells.

MATERIALS AND METHODS

Cell Lines

Human tumor cell lines HT-29 (9) and SW-1417 (J. Fogh, personal communication) were obtained from Dr. Jorgen Fogh. The LS-174T - Cl 10-1 cells were from a clone of human colonic tumor cells (10,11) given to us by Dr. Lynne P. Rutzky. All three tumor cell lines were derived from resected primary colon adenocarcinomas of female patients. FeInt cells were obtained from an aborted female human embryo. These epithelial cells had

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a normal diploid karyotype and did not induce tumor formation in any of ten mice inoculated intracranially with 2 million cells.[5]

Cell Culture

All cells were propagated in L-15 medium (Grand Island Biological Company, NY) containing 10% fetal calf serum (Reheis Chemical Company, Phoenix, AZ). Periodic tests for *Mycoplasma* contamination were negative. The studies described in this paper were performed on cells passaged at the following tissue culture generations: HT-29 (132–166); SW-1417 (17–24); LS-174T - Cl 10-1 (11–18), and FeInt (17–28).

Single-cell suspensions of HT-29 and FeInt cells were obtained after incubating the cells at 37°C with 0.05% trypsin-0.02% ethylenediamine tetraacetic acid (Grand Island Biological Company). For the SW-1417 and LS-174T - Cl 10-1 lines, single-cell suspensions were obtained after digestion at 37°C with an enzyme mixture containing 0.1% protease from *Streptomyces griseus*; 7000 units/g (Boehringer Mannheim Biochemicals, Indianapolis, IN); 200 units/ml of bovine testis hyaluronidase (Type IV-S from Sigma Chemical Company, St. Louis, MO); and 50 μg/ml of bovine pancreas DNAse (Sigma Chemical Company). All cells were resuspended in L-15 medium containing 10% fetal calf serum and were plated in tissue culture flasks (25 cm^2). The plating efficiency, determined as the fraction of cells which formed colonies of ⩾ 30 cells, was similar (4%–7%) for all four cell lines as was the doubling time of cell growth under these conditions (1.4–1.8 days). For drug cytotoxicity tests, fresh medium containing drug or no drug (controls) was added 2 days after plating. In most experiments, the cytotoxicity of OHUrd and of either FUra or FdUrd was determined for FeInt cells and for at least one tumor cell line simultaneously. After incubation for 3–5 days, the drugs were removed, fresh medium was added, and colonies were allowed to form. Cell viability relative to control was calculated as the fraction of colonies in treated flasks compared to control flasks. Each experiment was conducted at least twice.

Chemicals

OHUrd and OHUra were obtained from Sigma Chemical Company. High-performance liquid chromatography analysis showed that the OHUrd was 99% pure. A peak which cochromatographed with OHUra accounted for 0.4% of the ultraviolet absorbing material, and 0.6% of the material was not identified. Samples were analyzed with a Beckman Instruments, Inc (Fullerton, CA) Model 324 Liquid Chromatograph using an Ultrasphere ODS (25 cm × 4.6 mm, inner diameter) column. Chromatographs were obtained at ambient temperature with 20 mM sodium acetate, pH 4.5, as the eluting solvent. The flow rate was 1.5 ml/minute, and absorbance was monitored at 254 nm. FUra and FdUrd were obtained from Hoffmann-La Roche, Inc (Nutley, NJ).

[5]Giovanella BC. Manuscript in preparation.

RESULTS

The cytotoxic effects of OHUrd and of FUra on the colonic tumor cells compared to the FeInt cells are shown in figures 1 and 2. OHUrd was much more cytotoxic to all three tumor cell lines compared to the normal cells. FUra, on the other hand, was more cytotoxic to the FeInt cells. FdUrd was slightly more toxic to HT-29 cells than to FeInt cells (fig 3) and was more toxic to the LS-174T - Cl 10-1 cells than to the FeInt cells but less toxic to the SW-1417 cells (data not shown).

Further studies on the cytotoxic mechanism of OHUrd were conducted using the HT-29 cells. Coadministration of uridine or cytidine completely protected HT-29 cells from the cytotoxic effects of OHUrd, but deoxyribonucleosides afforded very little or no protection (table 1). Purine nucleosides were also ineffective (data not shown). Much higher concentrations of OHUra than OHUrd were required to decrease the viability of HT-29 cells, and this effect was not prevented by uridine (table 2) or by uracil (table 3).

DISCUSSION

The relative effects of cytotoxic treatment on neoplastic and nonneoplastic cells of the same histologic origin have been studied in our laboratory (12–14) to develop agents which specifically target tumor cells. A major dose-limiting toxic effect of several anticancer

TABLE 1.—Effect of pyrimidine nucleosides on the cytotoxicity of OHUrd to HT-29 cells*

Treatment	Cell viability (fraction of control)
OHUrd (250 μM)	0.007 ± 0.001
OHUrd (250 μM) plus:	
Uridine (5 μM)	0.60 ± 0.05
Uridine (40 μM)	1.13 ± 0.04
Cytidine (5 μM)	0.94 ± 0.11
Cytidine (40 μM)	1.08 ± 0.08
Deoxyuridine (200 μM)	0.008 ± 0.004
Deoxyuridine (1 mM)	0.022 ± 0.012
Deoxycytidine (200 μM)	0.009 ± 0.001
Deoxycytidine (1 mM)	0.013 ± 0.003
Thymidine (200 μM)	0.048 ± 0.014

* HT-29 cells were incubated with OHUrd ± nucleoside for 3 days, and cell viability was determined as described in the Materials and Methods section. Control cells were incubated with medium only. Nucleosides at these concentrations had no effect on cell viability. Values = mean ± SE.

TABLE 2.—Effect of OHUra and OHUra + uridine on HT-29 cells*

Treatment	Cell viability (fraction of control)
OHUra (2.5 mM)	0.09 ± 0.02
OHUra (1.0 mM)	0.42 ± 0.09
OHUra (0.4 mM)	1.11 ± 0.04
OHUra (1 mM) + uridine (1.0 mM)	0.44 ± 0.03

* HT-29 cells were incubated with OHUra or OHUra + uridine for 3 days, and cell viability was determined as described in the Materials and Methods section. Control cells were incubated with medium only. Values = mean ± SE.

TABLE 3.—Effect of OHUra and uracil on HT-29 cells*

Treatment	Cell viability (fraction of control)
OHUra (1.5 mM)	0.21 ± 0.04
Uracil (0.4 mM)	0.99 ± 0.01
Uracil (1.0 mM)	0.97 ± 0.02
Uracil (2.5 mM)	0.96 ± 0.01
OHUra (1.5 mM) plus:	
Uracil (0.1 mM)	0.20 ± 0.02
Uracil (0.4 mM)	0.19 ± 0.02
Uracil (1.0 mM)	0.17 ± 0.01
Uracil (2.5 mM)	0.28 ± 0.01

* Values = mean ± SE.

drugs is damage to the proliferating cells of the intestinal mucosa. For this reason, it would be of particular interest to investigate drugs which are relatively nontoxic to these cells. In these experiments, three colon adenocarcinoma cell lines were compared with normal cells derived from fetal intestine. All four of these human cell lines were epithelial. To our knowledge, there are no other reports of long-term cell cultures derived from normal human intestinal epithelium. The tumor cells were aneuploid and formed tumors in nude mice, whereas the FeInt cells had a normal human karyotype and did not cause tumor formation in these mice. Because many chemotherapeutic agents are more toxic to cells that divide rapidly, we selected for comparison cells which

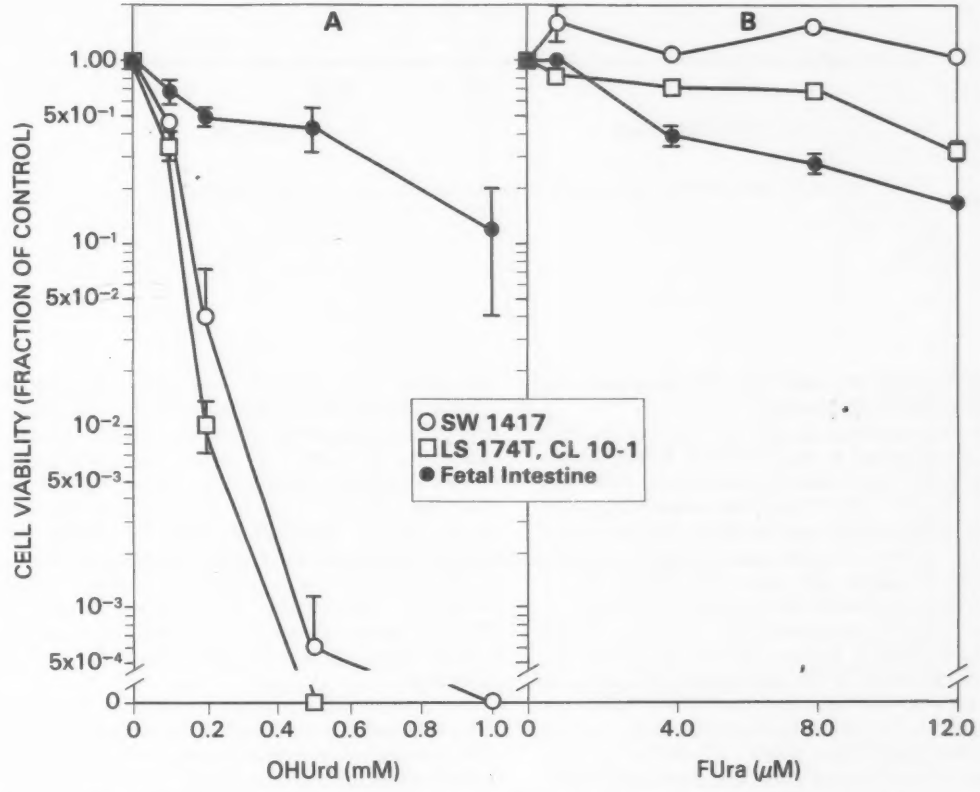

FIGURE 1.—Effect of OHUrd (Panel A) or FUra (Panel B) on viability of SW-1417 (○), LS-174T - Cl 10-1 (□), and FeInt (●) cells. Cell viability was determined as described in the Materials and Methods section.

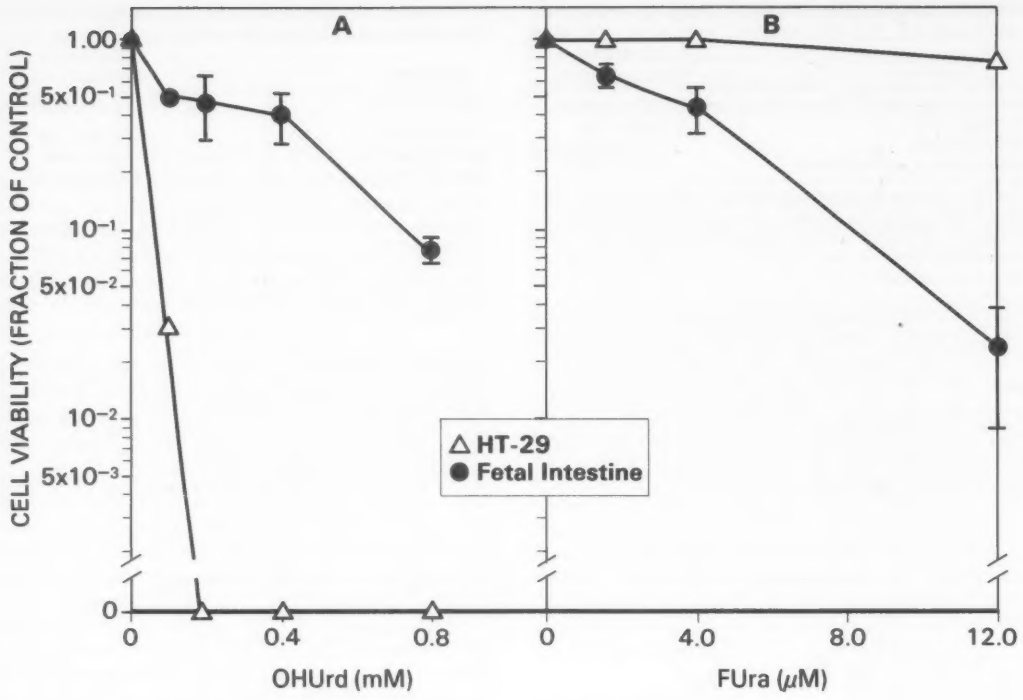

FIGURE 2.—Effect of OHUrd (Panel A) or FUra (Panel B) on viability of HT-29 (△) and FeInt (●) cells.

had approximately the same rate of division under the conditions of these experiments.

OHUrd was substantially more cytotoxic to the three tumor cell lines than to the FeInt cells. In comparison, the tumor cells were relatively resistant to FUra, and only the LS-174T - Cl 10-1 cells were substantially more sensitive to FdUrd than were the FeInt cells. The lack of specificity of FUra for these tumor cells is not unexpected. Most patients with colon carcinoma do not respond to FUra, and destruction of the proliferating intestinal mucosa is a major dose-limiting toxic effect for this drug and for FdUrd. Further investigation will be required to ascertain if the differential sensitivity to OHUrd signifies biochemical or other cellular differences which might be exploitable for improved chemotherapy. The results reported here suggest that OHUrd could be an effective agent against colon adenocarcinoma unless toxicity to other vital normal cells was dose-limiting. In vivo studies will be required to help answer this question.

Initial biochemical studies with HT-29 cells indicate that OHUrd antagonized pyrimidine nucleotide metabolism. The cytotoxicity of OHUrd was prevented by uridine or cytidine. Compared to OHUrd, OHUra was much less cytotoxic to HT-29 cells, and this effect was not reversed by uridine or by uracil. By analogy with data obtained from other systems, inhibition of enzymes on the de novo pyrimidine synthetic pathway, such as orotidylate decarboxylase (7), could be targets for OHUrd. Cofactor analogs derived from 5-hydroxyuridine 5′-triphosphate (15) may also play a role in the observed cytotoxicity.

These results indicate that comparison of drug sensitivity for colon tumor cells compared to FeInt cells may offer a method of screening potential anticancer agents that selectively kill these tumor cells.

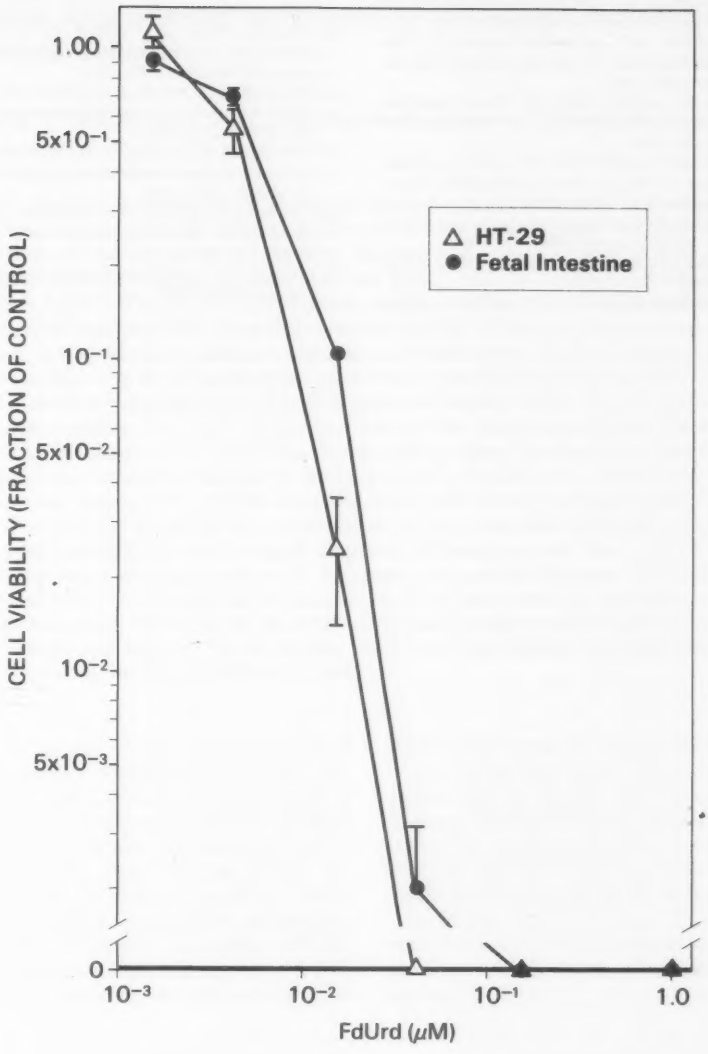

FIGURE 3.—Effect of FdUrd on the viability of HT-29 (△) and FeInt (●) cells.

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Deoxyguanosine Enhancement of Cytarabine Nucleotide Accumulation in Human Leukemia Cells[1,2,3]

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We studied the effect of deoxyguanosine (dGuo) on cellular cytarabine (ara-C) nucleotide accumulation of the human leukemia cell line K562 and of bone marrow blast cells derived from patients with acute nonlymphocytic leukemia. Exposure of cells in culture to dGuo increased ara-C nucleotide accumulation measured in cell lysate, with an average increase of 386% (range, 242%–537%) of control in the presence of 500 μM dGuo. Maximal elevation occurred after 8 hours of exposure and remained constant through 48 hours. dGuo also enhanced deoxycytidine nucleotide accumulation, but dGuo enhancement favored accumulation of ara-C nucleotides over dCyd nucleotides. In cell cycle kinetic studies using flow cytometry, dGuo slowed accumulation of cells with apparent S-phase DNA content in a concentration-dependent fashion. However, neither the rate nor the magnitude of this effect correlated with the increase in ara-C nucleotide accumulation. Since the increase in ara-C nucleotide accumulation caused by dGuo could be prevented by 5 μg/ml of cycloheximide, this process appears to require new protein synthesis. Although these data suggest that the elevation of ara-C nucleotide accumulation caused by dGuo may represent induction of enzyme synthesis, other possibilities are discussed. Exposure of bone marrow blast cells obtained from patients with acute nonlymphocytic leukemia to dGuo for 16 hours in liquid culture also increased ara-C nucleotide accumulation. In six of seven studies, exposure to dGuo in concentrations from 50 to 500 μM increased ara-C nucleotide accumulation from 160% to 3400%. These data suggest that dGuo may alter ara-C metabolism in a clinically useful fashion. [Cancer Treat Rep 69:851–857, 1985]

Cytarabine (ara-C) is one of the most useful antileukemic agents in current clinical practice (1–3). To be active, this important agent must be phosphorylated to ara-CTP (4–6). Recently, considerable interest has been generated in augmenting the ability of leukemic cells to form and retain ara-CTP, either with agents that inhibit deamination of ara-C such as tetrahydrouridine (THU) (7–9) or with agents that promote phosphorylation of ara-C such as thymidine or uridine (10–12). Additionally, the prospect of augmenting the ara-CTP pool by dramatically increasing the extracellular concentration of ara-C is being investigated (13).

We (14,15) and others (16) have observed that the purine deoxynucleoside dGuo augments ara-C toxicity in vitro. This enhancement has been correlated in K562 cells with an increased intracellular ara-CTP pool (15). Since in some systems deoxyguanosine (dGuo) is a substrate for dCyd kinase (NTP: deoxycytidine 5′-phosphotransferase, E. C. 2.7.1.74) (17–24), we investigated whether the observed increase in phosphorylation of ara-C can result from enhancement of dCyd kinase activity by dGuo.

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MATERIALS AND METHODS

Chemicals

5-(^{3}H)-ara-C (specific activity, 15.5 Ci/mmol) was purchased from Amersham/Searle, Arlington Heights, IL. 5-(^{3}H)-dCyd (specific activity, 26.8 Ci/mmol) was purchased from New England Nuclear, Boston, MA. ATP, phosphoenolpyruvate, ara-C, dCyd, and pyruvate kinase were obtained from Sigma Chemical Co, St. Louis, MO. THU was received as a gift from Leonard Kedda, Drug Development Branch, National Cancer Institute, Bethesda, MD.

Cell Culture

Culture conditions for the human leukemia cell line K562 have been described in detail (25). Log-phase growth cells at concentrations of 500,000 to 1 million cells/ml were used in all assays.

Bone marrow mononuclear cells from patients were isolated and cultured as follows: 4 ml of bone marrow/patient was aspirated from the posterior iliac crest into syringes coated with 0.5 ml of preservative-free heparin, 1:1000. Mononuclear cells were isolated on a Ficoll-Hypaque gradient (26) and washed three times in phosphate-buffered saline, pH 7.4. Prior to the second wash, cells were incubated in 0.74% ammonium chloride for 10 minutes to lyse contaminating erythrocytes. Final erythrocyte contamination was always < 5% of leukocyte count. Trypan blue-determined viability of bone marrow mononuclear cells, of which > 85% were myeloblasts, was routinely > 90% after isolation and washing. After washing, mononuclear cells were resuspended at a cell concentration of 1 million/ml in Roswell Park Memorial Institute 1640 medium supplemented with 10% heat-inactivated (56°C, 35 minutes) fetal calf serum; L-glutamine (2 mM); penicillin (50 IU/ml); and streptomycin (50 μg/ml); with or without dGuo. Cell suspensions were incubated in 25-cm^2 tissue culture flasks (Falcon Plastics, Oxnard, CA) at 37°C, 7.5% CO_2, for 16 hours prior to assay.

Preparation of Cell Extracts

K562 cells were incubated with dGuo in 25-cm^2 tissue culture flasks at 37°C, 7.5% CO_2, prior to assay. At designated times, cells were cooled on ice for 5 minutes, then centrifuged at 400 × *g* for 10 minutes at 4°C. Sedimented cells were washed twice in ice-cold phosphate-buffered saline, pH 7.4, which removed > 99.9% of dGuo. Following the washing, sufficient ice-cold distilled water was added to the cell sediments to obtain a final concentration of 1 million cells/20 μl of water. Cell lysis was accomplished by four freeze-thaw cycles. Following clarification of cell lysates by centrifugation (20,000 × *g* × 15 minutes, 4°C), 20 μl of supernatant solutions was added immediately to reaction mixes for phosphorylating activity assay.

Kinase Activity Assay

Kinase activity was assayed by modification of the method of Coleman et al (22). The reaction mixture contained 20 μl of cell extract; 60 mM Tris-HCl (pH 7.8); 18.8 mM KCl; 15 mM KF; 2 mM dithiothreitol; 0.4 mg/ml of bovine serum albumin; 6 mM ATP; 7.2 mM $MgCl_2$; 0.4 mM THU; 6 mM phosphoenolpyruvate; 10.4 units/ml of pyruvate kinase; either 0.2 mM ara-C or 0.2 mM dCyd; and 1.6 μCi of ^{3}H-ara-C (specific activity, 15.5 Ci/mmol) in a final volume of 0.1 ml. The concentrations of substrates used (ara-C or dCyd and ATP) were shown in preliminary experiments to be sufficient to cause the reaction to exhibit zero-order kinetics with respect to substrate concentration (saturating conditions). Reactions were incubated at 37°C for 30 minutes. We have determined that formation of phosphorylated product increases linearly through 30 minutes (data not shown). Following reaction, 40 μl of reaction mix was applied to prewashed DE-81 anion-exchange discs (Whatman Inc, Clifton, NJ). Nonphosphorylated reactants were washed off the discs by immersion in a 2:1 concentration of 1 mM ammonium formate for 5 minutes, followed by four successive washes in a 1:1 concentration of 1 mM ammonium formate. After the washes, the discs were placed in liquid scintillation vials with 400 μl of 0.2 mM Tris-HCl, pH 7.5, for 1 hour at room temperature, followed by 5 ml of Ready-Solv (Beckman Instruments Inc, Fullerton, CA) scintillation solution. Radioactivity was determined on a Searle Mark III scintillation spectrometer (Amersham/Searle). The amount of nucleotide accumulation was expressed as units (1 unit = 1 pmol of nucleotide formed/minute/10^6 cells).

Flow Cytometry

Logarithmically growing cells (500,000–700,000/ml) were incubated in the presence of appropriate concentrations of dGuo for periods of up to 48 hours, then collected by sedimentation (400 × *g* for 10 minutes) and stained in ice-cold propidium iodide (0.05 mg/ml) (Sigma Chemical Co, St. Louis, MO) in 0.1% sodium citrate, according to the method of Krishan (27). DNA content per cell nucleus was determined by measurement of red fluorescence (excitation wavelength = 488 nm) using flow cytometry (FACS IV, Becton Dickinson, Mountain View, CA) analyzed and stored via the on-line computer system of the FACS IV. Cell cycle-phase distribution was determined as described previously (14).

RESULTS

dGuo enhanced ara-C nucleotide accumulation in K562 cells (figs 1 and 2). Increased ara-C nucleotide accumulation was noted after 8 hours of incubation with 100 or 500 μM dGuo and was sustained through 48 hours (fig 1). The data shown (figs 1 and 2) are the combined results of multiple experiments. We compared simultaneously the effects of dGuo on ara-C and dCyd nucleotide accumulation in K562 cells (fig 2). dGuo stimulated both ara-C and dCyd nucleotide accumulation. At concentrations of dGuo up to 100 μM, both ara-C and dCyd nucleotide accumulation were stimulated in a clearly dose-dependent fashion; however, dCyd nucleotide accumulation stimulation was less marked than for ara-C. Exposure to 500 μM dGuo did not cause further stimulation of dCyd nucleotide accumulation. Therefore, as the concentration of dGuo increased, accumulation of ara-C nucleotide was favored over dCyd.

Cycloheximide (5 μg/ml) suppressed the incorporation of labeled amino acid into acid-insoluble material > 95%, while causing only a 6% drop in cell viability after 16 hours of incubation (data not shown). Therefore, incubation of K562 cells with 5 μg/ml of cycloheximide plus dGuo was used to determine if the increase in ara-C phosphorylating activity was dependent on new protein synthesis. Cycloheximide diminished the increase in ara-C nucleotide accumulation caused by 500 μM dGuo to a statistically insignificant value ($P = 0.001$ for control vs 500 μM dGuo alone; $P = 0.2$ for control vs 500 μM dGuo plus 5 μg/ml of cycloheximide).

Addition of 1 μM hydroxyurea, an ara-C dephosphorylation inhibitor (28), to the reaction mix did not significantly alter ara-C nucleotide accumulation.

We investigated the effects of dGuo on K562 cell cycle-phase distribution, since the biochemical processes requisite for ara-C phosphorylation are found only in S-

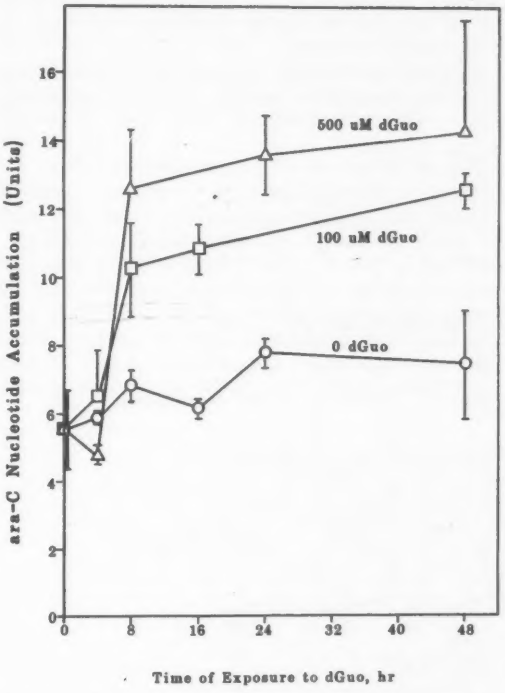

FIGURE 1.—Time-course of enhancement of ara-C nucleotide accumulation in K562 cells by dGuo. Ara-C nucleotide accumulation was assayed after incubation with 0 μM (○), 100 μM (□), or 500 μM (△) dGuo for time indicated. Assays were carried out in triplicate, as described in the Materials and Methods section. The data shown are combined results of 2 separate experiments. 1 unit of activity = 1 pmol of phosphorylated product formed/min/10^6 cells. Vertical bars = SEM.

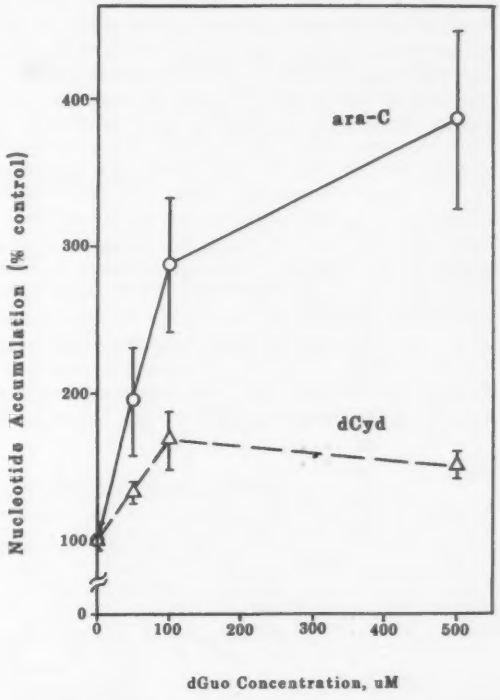

FIGURE 2.—Effects of dGuo on ara-C or dCyd phosphorylation. K562 cells were exposed to varying concentrations of dGuo in vitro for 16 hrs, after which cell-free extracts were prepared and assayed for ara-C nucleotide accumulation (○———○) or dCyd nucleotide accumulation (△---△), as described in the Materials and Methods section. Data shown are combined results of 3 separate experiments. Control values (no dGuo) for ara-C nucleotide accumulation were 3.6, 5.6, and 5.8 units. Control values for dCyd nucleotide accumulation were 9.6, 12.6, and 62.7 units. Vertical bars = SEM.

phase cells (23) (fig 3). Since dGuo significantly alters intracellular pools of deoxynucleoside triphosphate (25), the percentage of cells in S phase cannot be estimated reliably in cells exposed to dGuo using ^{3}H-dThd labeling indices or by such techniques as specific S-phase labeling, which quantifies the percentage of cells which incorporate bromodeoxyuridine into DNA (29). For this reason, we investigated the effects of dGuo on the percentage of cells with S-phase DNA content, using flow cytometry. Figure 3 shows the results of a typical experiment. Similar to our findings with HL-60 cells (14), exposure of K562 cells to dGuo caused a concentration-dependent slowing of cell progression through S. dGuo (100 μM) caused a cohort of cells to enter and to progress into the early to mid-S DNA content region by 48 hours; this is shown by a gradual broadening of the postmitotic (G_0/G_1) peak with time, beginning at 8–16 hours, with most of the cells moving out of the G_0/G_1 region and into the S region by 48 hours. In contrast, 500 μM dGuo inhibited cell progression through S to a greater extent, since after 48 hours the cohort of cells was able to reach only the early S region, as evidenced by only a slight broadening of the postmitotic peak and a smaller percentage of cells in S at this time, compared to 100 μM dGuo. Cell growth declined comparably, however, in cultures containing either 100 or 500 μM dGuo, with cell numbers approximately 45% of those of control cultures at 48 hours. Note that addition of medium without dGuo to the controls also caused a slight perturbation of cell cycle-phase distribution, with a small cohort of cells passing through the early and mid-S regions at 8 and 16 hours, respectively. At 8 hours, the time at which dGuo enhancement of ara-C phosphorylation reaches maximal levels, perturbations of cell cycle-phase distribution caused by exposure of cells to 100 or 500 μM dGuo were minimal compared to control (fig 3).

Thymidine, a pyrimidine nucleoside capable of causing prolongation of cell transit through S phase (14), also caused an increase in ara-C nucleotide accumulation, although quantitatively less than that caused by dGuo. Using linear regression analysis, the increment in ara-C nucleotide accumulation caused by a given concentration of dThd was directly proportional to the increment in the flow cytometric estimate of the percentage of cells in S caused by dThd (correlation coefficient, $r^2 = 0.999$; slope of 1.05% change in ara-C nucleotide accumulation per percent change in cells in S). In contrast, no similar correlation was observed with dGuo ($r^2 = 0.007$).

By using bone marrow blast cells from patients with acute nonlymphocytic leukemia, we investigated the

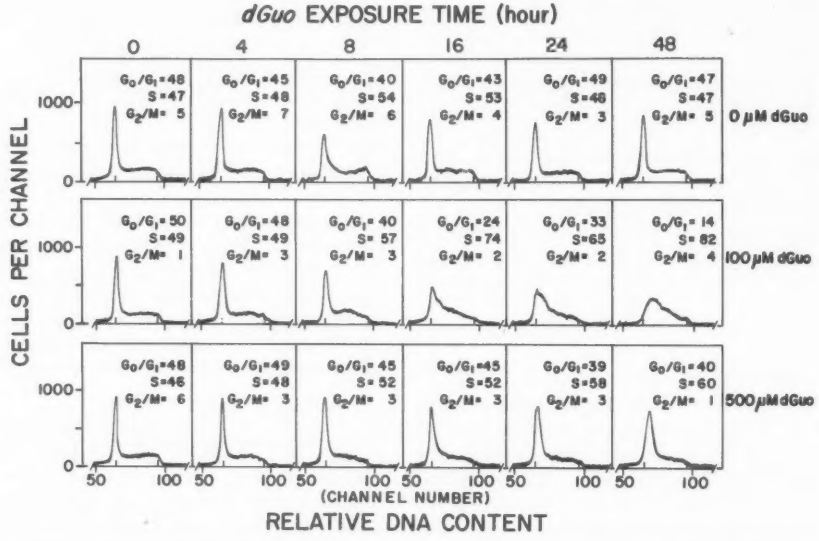

FIGURE 3.—Change in DNA content distribution histogram of K562 cells at designated times after addition of 0, 100, or 500 μM dGuo to culture medium. DNA content was measured by quantification of red fluorescence of cell nuclei stained with propidium iodide using flow cytometry (*see* Materials and Methods section). Ordinate of each histogram represents No. of cells at each fluorescence intensity. Fluorescence intensity is represented on the abscissa. Intensity increases from left to right. Cell cycle-phase distribution (% of cells in either G_0/G_1, S, or G_2/M) was determined by electronic gating, as described in the Materials and Methods section. Tick marks under each histogram represent the peak channels of the G_0/G_1 and G_2/M populations in unperturbed cells. Shown are the results of a typical experiment.

effect of 16-hour exposures to dGuo on ara-C nucleotide accumulation by leukemic blast cells (table 1). Baseline ara-C nucleotide accumulation by cells of all patients but one (No. 6) was considerably lower than that of K562 cells (cf fig 2), varying from 0.07 to 5.3 units. This range of activity is in agreement with that reported by Coleman et al (22), who assayed dCyd kinase activity of human myeloblasts. In six of seven patients studied, dGuo enhanced ara-C nucleotide accumulation (table 1). In four of these patients (Nos. 1, 2, 3A, and 6), maximal enhancement ranged from 160% to 310% of control. In two patients (Nos. 3B and 5), however, in whom the baseline nucleotide accumulation was extremely low, maximal enhancement reached 900% and 3400%, respectively.

DISCUSSION

In some cell systems, dGuo will serve as a substrate, albeit a weak one, for dCyd kinase (17–20), the enzyme responsible for ara-C phosphorylation (21–24). The goal of our study was to investigate whether dGuo, a relatively high Km substrate for dCyd kinase, could enhance this enzyme activity in leukemic cells, so that phosphorylation of the potent antileukemia agent ara-C would be promoted. This possibility is especially intriguing, since dGuo augments ara-C cytotoxicity (14–16).

A number of mechanisms could account for the dGuo-mediated increase in ara-C nucleotide accumulation. These mechanisms will now be considered individually.

(*a*) dGuo may inactivate or repress synthesis of enzyme(s) which degrade ara-C or ara-C nucleotides. We feel that these mechanisms are unlikely explanations of the effect of dGuo on ara-C nucleotide accumulation. Addition of THU, an inhibitor of dCyd-deaminase (E. C. 3.5.4.1) to the reaction mix, should eliminate the influence of fluctuations in Cyd deaminase activity on ara-C nucleotide accumulation (7–9, 30). Similarly, although dephosphorylation has been shown to be a major influence on ara-C nucleotide pool size (28), phosphatase activity is routinely inhibited in our assay by the addition of KF to the reaction mix. We verified inhibition of phosphatase activity by observing no change in ara-C nucleotide accumulation with the addition of hydroxyurea, which inhibits ara-C dephosphorylation (28).

(*b*) dGuo may cause changes in nonprotein cellular metabolites, which regulate ara-C phosphorylation. We considered this possibility because we (21) and others (16) have shown that dGuo diminishes the intracellular pool of dCTP. dCTP is a powerful inhibitor of dCyd kinase (23,24). It is likely that the diminished intracellular dCTP pool contributes to increased ara-CTP formation in the presence of dGuo in the intact cell. However, in our experiments with cell-free extracts, elevation of ara-C nucleotide accumulation by dGuo appears to require new protein synthesis, since the addition of cycloheximide blocks the increase in enzyme activity. This suggests that increased ara-C phosphorylation caused by dGuo is not due simply to an alteration in the concentrations of cellular metabolites that regulate the activity of dCyd kinase. However, alternate explanations exist for the blocking effect of cycloheximide, such as inhibition of protein synthesis, which interferes with progression through the cell cycle or which blocks intracellular transport of dGuo.

(*c*) The S-phase dependence of cytosolic dCyd kinase activity suggests the possibility that increased ara-C nucleotide accumulation caused by dGuo is a by-product of its effect on cell cycle distribution. Flow cytometric data suggest that dGuo-mediated S-phase accumulation does not correlate temporally with increased ara-C nucleotide accumulation. However, S phase is not homogenous biochemically; it is possible that cytosolic dCyd kinase activity is concentrated early in S phase.

TABLE 1.—Ara-C nucleotide accumulation in acute nonlymphocytic leukemic bone marrow blast cells*

Patient No.	French-American-British classification	Ara-C nucleotide accumulation (units)			
		0 μM dGuo	50 μM dGuo	100 μM dGuo	500 μM dGuo
1	M4	1.2	—	2.1	3.0
2	M2	0.9	2.8	1.0	1.3
3A†	M3	0.7	0.7	1.9	0.6
3B†	M3	0.07	2.4	1.6	1.6
4	M2	0.9	0.9	0.7	0.9
5	M6	0.1	0.9	0.8	0.5
6	M2	5.3	8.5	7.4	6.4

*Units of ara-C nucleotide accumulation activity (1 unit = 1 pmol of product formed/min/10^6 cells). Cell harvesting, incubation conditions, and assays were carried out as described in the Materials and Methods section. SD = 15% of mean value for all data points.

†Patient was analyzed twice, before (A) and after (B) failed chemotherapeutic attempt. Chemotherapy did not include ara-C.

Since it is difficult to distinguish late G_1 from early S cytofluorometrically, even with sophisticated computer mapping programs, it is possible that the number of cells in early S phase could be incremented sufficiently by 8 hours of exposure to 100–500 μM dGuo to contribute to augmented ara-C nucleotide accumulation, without the increment being detected cytofluorometrically. Therefore, we cannot exclude a contribution of cell cycle perturbations to dGuo-mediated ara-C nucleotide accumulation augmentation, although we point out that dThd, under analytic conditions identical to those used for dGuo, caused an increment in ara-C nucleotide accumulation exactly in proportion to its effect on increasing S-phase cells. No such relationship was observed with dGuo, adding some weight to the view that cell cycle perturbation cannot entirely account for the effects of dGuo.

(*d*) dGuo may induce the synthesis of the enzyme(s) responsible for ara-C phosphorylation. On the basis of the data presented, we feel that this is certainly a possibility; however, one would expect the magnitude of increase in ara-C or dCyd nucleotide accumulation to be considerably greater than that observed if dGuo caused true induction of cellular synthesis of dCyd kinase. dCyd kinase induction also does not explain the favoring of ara-C nucleotide accumulation over dCyd nucleotides. Several hypotheses for this observation can be entertained, including, among others: (*a*) elevation of dCMP deaminase (E. C. 3.5.4.b) activity, causing preferential conversion of dCMP to dUMP, which may be partially dephosphorylated, or (*b*) induction of an enzyme which preferentially phosphorylates ara-C. All of these possibilities are entirely speculative at this time, since the data presented herein do not allow us to distinguish among them.

The enhancement of ara-C phosphorylating activity in human leukemia cells by dGuo appears not to be limited to a particular cell line, ie, K562. In marrow blast cells from five of six patients with acute nonlymphocytic leukemia, dGuo stimulated ara-C phosphorylation. As expected, the control (no dGuo) values of ara-C phosphorylating activity exhibited a large interpatient coefficient of variation (138% for all seven patients and 71% if extremely high value of Patient 6 is excluded). A large coefficient of variation of ara-C phosphorylating activity has been observed in several previous studies of ara-C phosphorylating activity in patient blast cells (31–34). The magnitude of patient blast cell ara-C phosphorylating activity enhancement by dGuo was comparable to that observed for K562 cells. However, the dose-response relationship was not as clear-cut as with K562, since the ara-C phosphorylating activities of several patients were maximally enhanced by 50 μM dGuo and declined at higher concentrations. This variability of response of ara-C phosphorylating activity to dGuo may reflect interpatient variation in susceptibility to dGuo-mediated disturbances in cell cycle kinetics. The data presented in this paper as well as those of others suggest that high concentrations of purine nucleosides may prevent entry of cells into S phase (35), the phase in which ara-C phosphorylating activity increases dramatically (23). It is possible that patient blast cell ara-C phosphorylating activity may decline somewhat at very high dGuo concentrations because stimulation of ara-C phosphorylating activity is counterbalanced by dGuo-mediated interference with entry into and progression through S phase.

The fact that dGuo increased phosphorylating activity in bone marrow leukemic blast cells from five of six patients suggests that the phenomenon may be a general one among human leukemic cells; thus, the possibility that dGuo may be a clinically important modifier of ara-C activity is suggested. Also appealing is the fact that high concentrations of dGuo favor ara-C phosphorylation over dCyd phosphorylation. The antitumor effect of ara-C is correlated positively with ara-CTP pool size (4,5,10,31–34,36) and negatively with dCTP pool size (23,37,38). Phosphorylation of circulating plasma dCyd may be protective against ara-C toxicity (39–41). The enhancing effect of dGuo may promote a favorable ara-CTP/dCTP pool size ratio and reduce the protective effect of dCyd in malignant cells.

These data support a growing body of evidence that dGuo is an important modifier of ara-C activity (14–16). We are currently investigating the animal pharmacology of dGuo to determine whether plasma concentrations necessary for enhancing of ara-C phosphorylation can be achieved and sustained without excessive toxicity.

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Acute Effects of Doxorubicin on Chronotropic and Inotropic Mechanisms in Guinea Pig Atria[1,2,3]

P. M. Politi, R. P. Rothlin, and J. E. B. Pinto[4,5,*]

This study was designed to test the possible acute effects of doxorubicin (DOX) on isolated guinea pig atria incubated in Locke's solution. Different concentrations of DOX (10^{-6} to 10^{-4} M) were added to the medium 30 minutes before the concentration-response curves to noradrenaline and histamine were carried out. DOX (10^{-4} M) significantly reduced spontaneous atrial rate. Atropine (10^{-6} g/ml) was unable to modify this cardiodepressant effect. DOX (10^{-4} M) produced a competitive beta blocking effect, shifting to the right the concentration-response curve to noradrenaline without altering the maximal chronotropic response. On the other hand, this anthracycline (10^{-4} M) not only antagonized chronotropic responses to histamine, but significantly reduced the maximal effect mediated by this amine. Isolated left guinea pig atria electrically paced were used to determine the effects of DOX on positive inotropic activity promoted by noradrenaline and histamine. Similarly to what was observed in chronotropic experiments, DOX (10^{-4} M) produced a competitive beta blocking action and a noncompetitive inhibition of the positive inotropic action developed by histamine. Lower concentrations of DOX failed to modify the chronotropic responses to both amines. However, after 60 minutes of incubation with DOX, 10^{-5} M of this drug produced a shift to the right of the concentration-response curves to noradrenaline and histamine and depressed the maximal chronotropic response to these amines. These effects were not observed with 3×10^{-6} M DOX. These results are compatible with the idea that a nonspecific interaction of DOX with cardiac beta and histaminergic receptors could be involved in the acute cardiotoxic mechanism produced by this anthracycline. [Cancer Treat Rep 69:859–865, 1985]

Doxorubicin (DOX), an anthracycline antibiotic, is an important cytotoxic agent that has demonstrated activity against a wide spectrum of human neoplasms (1,2). Unfortunately, the therapeutic benefit of this drug is limited due to a severe, cumulative cardiotoxicity frequently associated with long-term use of this agent (3). Premature ventricular beats, supraventricular arrhythmias, and nonspecific abnormalities on the ECG are some of the acute cardiac effects induced by DOX (4). Chronic cardiotoxicity is one of the most worrisome side effects produced by this drug, and appears clinically as a dose-related cardiomyopathy with heart failure often resistant to digitalis (5). Several hypotheses have been postulated to explain DOX cardiotoxicity, including interaction with DNA (6), production of free radicals (7,8), membrane lipid damage (9,10), mitochondrial membrane effects (11), and modification of calcium transport (12,13). Among these hypotheses, it has been reported that histamine and catecholamine release could be related to DOX cardiotoxicity (14,15). Moreover, Dorr and Alberts (16) have recently shown that beta-adrenoceptors may be involved in DOX skin extravasation toxicity.

This study was undertaken to evaluate the possible effects of DOX on cardiac beta-adrenergic and histaminic mechanisms, and its potential role in developing acute cardiotoxic action.

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MATERIALS AND METHODS

Adult guinea pigs of either sex (300-400 g of body weight) were killed by a blow on the nape and bled by bilateral carotid section. The heart was immediately placed in Locke's solution (composition in mM: NaCl, 154; KCl, 5.6; $CaCl_2$, 2.2; Na_2HCO_3, 6; glucose, 11.1; EDTA, 0.04; and ascorbic acid, 0.11), which was kept at room temperature and bubbled with oxygen. The atria were carefully dissected and placed in an isolated organ bath containing continuously oxygenated Locke's solution at 37°C and pH 7.4. One end of the tissue strip was tied to a Grass force displacement transducer; the atrial rate was recorded on a Grass polygraph. The atria were allowed to stabilize until their spontaneous rate did not change by more than five beats per minute during one 10-minute period. This took about 1 hour; the bathing fluid was replaced every 10 minutes. DOX was added to the fluid 30 or 60 minutes before the performance of the concentration-response curve, and remained throughout the study.

For inotropic studies, the isolated left atrium was mounted in the organ bath, stretched up to 1 g of tension, and allowed to stabilize for 30 minutes before the addition of drugs. The atrium was electrically paced at 3 Hz with pulses 5 vol greater than threshold value. Concentration-response curves to *l*-norepinephrine and histamine were obtained in the spontaneously beating atria and in the electrically paced left atrium. The amines were added cumulatively in such a way that the final concentration in the bath was increased by a factor of three whenever a steady response to the previous concentration was achieved. Intervals between two successive concentrations were not > 3 minutes.

Reserpine pretreatment was carried out by administration of 5 mg/kg of the drug 24 hours before the experiments. In those studies where atropine was used, it was added to Locke's solution from the beginning of the experiments.

The following chemicals were employed: *l*-noradrenaline bitartrate monohidrate (Hoechst), reserpine phosphate, histamine dehydrochloride (Sigma), doxorubicin hydrochloride (Montedison-Farmitalia), and atropine sulfate.

Statistical analysis of data was performed with the Student's two-tailed t test or analysis of variance.

RESULTS

Effects of 30-Minute Incubation With DOX on Chronotropic and Inotropic Activity

DOX was tested for its ability to modify the rate of isolated spontaneously beating guinea pig atria. The effects of different concentrations of DOX are shown in figure 1. DOX at 3×10^{-6} M and 10^{-5} M did not modify

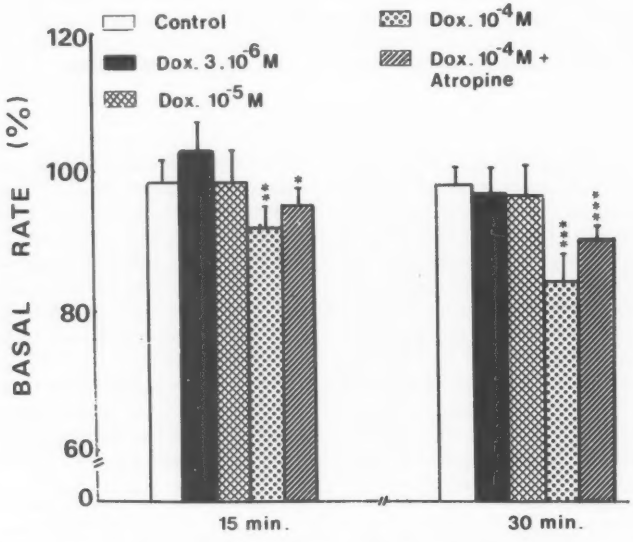

FIGURE 1.—Effect of DOX on spontaneous rate of isolated guinea pig atria. Atrial rate was recorded immediately before and after 15 and 30 mins of the addition of DOX (10^{-4} M) to the incubation medium. Atropine sulfate (10^{-6} g/L) was added to Locke's solution from the beginning of the corresponding experiment. Vertical bars represent mean ± SEM of the least 4 experiments. * = $P < 0.05$; ** = $P < 0.01$; and *** = $P < 0.001$.

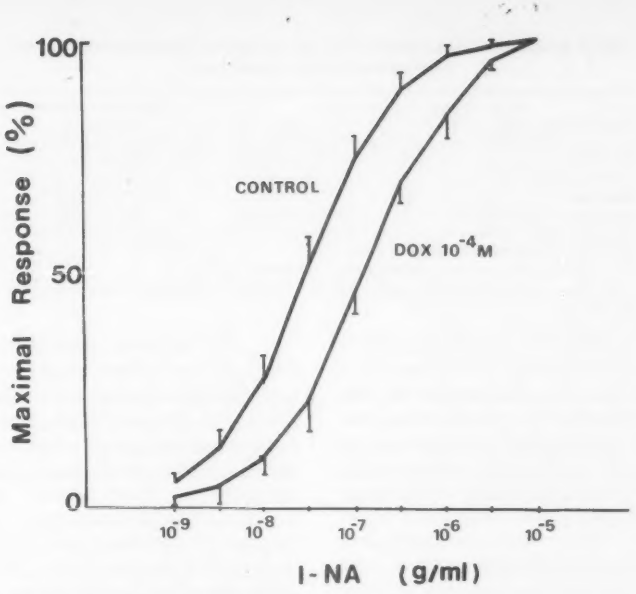

FIGURE 2.—Effect of DOX on positive chronotropic activity induced by *l*-noradrenaline (*l*-NA) in isolated guinea pig atria. DOX (10^{-4} M) was added to the incubation medium 30 mins before the concentration-response curves were done. Each point represents mean ± SEM.

atrial rate from controls (P = not significant). Only DOX at 10^{-4} M significantly depressed it after 15 minutes of incubation ($P < 0.01$). Neither previous reserpine pretreatment nor addition of atropine (10^{-6} g/ml) to Locke's solution modified the negative chronotropic activity of DOX at 10^{-4} M.

The effects of 30-minute incubation with DOX on the positive chronotropic response to *l*-noradrenaline are shown in table 1. It is evident from the concentration-response curves to noradrenaline that only 10^{-4} M DOX significantly modified the pD_2 value. This blocking effect is illustrated in figure 2, which shows that DOX (10^{-4} M) promoted a parallel shift to the right of the concentration-effect curve without modifying the maximal response to noradrenaline.

Since previous studies reported that DOX depressed chronotropic responses to a single concentration of histamine (17), it was of interest to test the effect of this anthracycline on histaminic cardiac activity. In contrast to the results observed with noradrenaline, 10^{-4} M DOX not only antagonized the responses to histamine, but also reduced the maximal chronotropic effect evoked by this amine (table 2). To discard the possible involvement of endogenous noradrenaline in the chronotropic responses to histamine, a series of experiments were carried out in reserpine-pretreated animals. Similar results were obtained in this experimental group (table 2).

Under these experimental conditions, 10^{-4} M DOX induced a depression of the maximal response to histamine which was of the same magnitude as that obtained in nonreserpinized guinea pigs (table 2).

TABLE 1.—Effects of 30-min incubation with DOX on positive chronotropic response to *l*-noradrenaline in guinea pig atria*

Experimental group	n	pD_2†	Maximal response (beats/min)
Control	5	7.059 ± 0.072	96 ± 7.1
3×10^{-6} M DOX	3	6.772 ± 0.150	101 ± 30.6
10^{-5} M DOX	4	6.850 ± 0.155	108 ± 17.9
10^{-4} M DOX	6	6.326 ± 0.071‡	94.6 ± 12.3

* Results are expressed as mean ± SEM.
†pD_2 = mean negative logarithm of molar ED_{50}.
‡$P < 0.01$.

TABLE 2.—Effects of DOX, exposed for 30 mins, on positive chronotropic response to histamine in guinea pig atria*

Experimental group	n	pD_2	Maximal response (beats/min)
Control	6	5.821 ± 0.078	107.3 ± 5.1
10^{-4} M DOX	4	5.939 ± 0.053	72.6 ± 8.2†
Reserpine	6	5.712 ± 0.133	117.8 ± 11.4
Reserpine + 10^{-4} M DOX‡	5	6.443 ± 0.105§	78.0 ± 6.5¶

*Results are expressed as mean ± SEM.
†$P < 0.001$ (difference from control group—analysis of variance).
‡Reserpine pretreatment was carried out by a 5-mg/kg sc injection 24 hrs before the experiments.
§$P < 0.05$.
¶$P < 0.01$.

Furthermore, the exposure of left atria for 30 minutes to 10^{-4} M DOX shifted to the right the concentration-response curve to noradrenaline without depressing the maximal inotropic response (table 3). As shown in this table, 10^{-4} M DOX significantly reduced the maximal response to histamine ($P < 0.02$).

Effects of a Longer Exposure to DOX

Since the acute effects described above were observed only with a high concentration of DOX, additional experiments were undertaken to test if a longer exposure to this drug could reduce the concentration of DOX required to produce these effects.

As shown in table 4, the previous addition of DOX (10^{-5} M) to the incubation medium for 60 minutes significantly depressed spontaneous chronotropic and inotropic activities. Moreover, 10^{-5} M and 10^{-4} M DOX not only produced a shift to the right of the concentration-response curve to noradrenaline and histamine, but also reduced the maximal chronotropic response to these amines (table 5).

DISCUSSION

The chronic cardiotoxic effects of DOX and other anthracycline agents have been extensively studied (18–21). Much less is known about the acute cardiac effects of these compounds. The clinical relevance of early anthracycline-induced cardiotoxicity has been stressed by Bristow et al (22), who reported that four types of acute cardiac manifestations developed after initial courses of anthracycline antitumor treatment. They recognized a pericarditis-myocarditis syndrome, a transient myocardial dysfunction sometimes leading to heart failure, atrial and ventricular rhythm disturbances, and possibly drug-induced coronary vasoconstriction. A wide spectrum of cardiac arrhythmias, detected through 24- and 48-hour Holter monitoring, have been reported during DOX administration (23,24). Moreover, some cases of acute myocardial injury (25) or sudden death (26) have been described.

Our results show an acute negative chronotropic action of DOX on spontaneously beating guinea pig atria. This effect does not seem to be due to acetylcholine release from parasympathetic nerve endings, since it is still present after addition of atropine to the incubation medium. A similar cardiodepressant action was observed in isolated rabbit atrioventricular nodes (27), which was possibly mediated by changes in potassium permeability. In addition, isolated rat myocytes exposed to DOX (10^{-5} M, 10^{-4} M) decreased spontaneous heart rate and stopped

TABLE 3.—Effects of 30-min incubation with 10^{-4} M DOX on the maximal inotropic activity induced by noradrenaline and histamine*

	Experimental group		
	Control n = 6	DOX n = 5	*P* value
Noradrenaline			
Maximal inotropic response†	132.6 ± 31.5	140.8 ± 18.8	NS‡
pD_2	6.529 ± 0.118	6.185 ± 0.057	0.05
Histamine			
Maximal inotropic response†	52.1 ± 6.3	26.2 ± 3.1	0.02

*Results are expressed as mean ± SEM.
†Expressed as % increase over baseline values.
‡NS = not significant.

TABLE 4.—Effects of 60-min incubation with DOX on the spontaneous chronotropic and inotropic activities of guinea pig atria.

Experimental group	% of baseline values ($\bar{x}$ ± SEM)	
	Chronotropic	Inotropic
Control	102.0 ± 1.5	96.2 ± 3.4
3×10^{-6} M DOX	100.7 ± 2.6	97.8 ± 4.7
10^{-5} M DOX	88.4 ± 7.4*	81.6 ± 5.7*
10^{-4} M DOX	81.1 ± 4.2*	54.9 ± 4.5†

*$P < 0.05$.
†$P \geq 0.01$.

beating after 7–36-minute average incubation (28).

Since beta-adrenergic receptor density has been found to be diminished in failing human hearts (29), and chronic DOX treatment in rats depressed the cardiac stimulant action of dobutamine (30), a series of experiments were undertaken to detect the possible DOX-beta-adrenergic receptor interaction in guinea pig atria. A significant beta blocking action was observed with 10^{-4} M DOX. This inhibitory effect seems to be of the competitive type, since DOX produced a parallel shift to the right of the concentration-response curve to noradrenaline without modifying the maximal chronotropic response. These results are compatible with an interaction between DOX and cardiac beta-adrenoceptors. A similar interaction has been suggested to explain acute toxic effects of DOX produced by skin extravasation. This anthracycline-induced injury could be partially prevented by propranolol or isoproterenol pretreatment, but not by the $beta_2$ agonist terbutaline (16,31). Furthermore, as shown in table 3, 10^{-4} M DOX promoted a negative inotropic effect on concentration-response curve to noradrenaline in electrically paced isolated guinea pig atria. This corroborates the competitive beta blocking action induced by this anthracycline in chronotropic studies.

Histamine release has been observed in animals treated with DOX, and combined adrenergic and histaminic blockade has prevented the myocardial damaging effects of this anthracycline (14,15). Moreover, chromolyn sodium pretreatment protected rabbits from cardiac toxic actions of DOX (32). These results prompted us to assess the effects of DOX on atrial histaminic mechanisms. Our results show an acute histaminic blocking action of 10^{-4} M DOX in guinea pig atria. This effect is compatible with a noncompetitive antagonism, since DOX depressed the maximal chronotropic response to histamine. These data corroborate previous reports showing a decrease of atrial response to a single concentration of histamine in guinea pigs treated ip with DOX (17). Similar results were obtained in reserpinized animals. These experiments allow us to discard the possible participation of an indirect adrenergic component mediating chronotropic responses when high concentrations of histamine are used. Furthermore, a noncompetitive histaminic blocking action of 10^{-4} M DOX was also observed when inotropic responses were studied.

Our results show that DOX cardiotoxicity depends on drug concentration and exposure time. This is in agreement with previous clinical studies which show a reduced cardiac DOX toxicity with prolonged, continuous iv infusions (33–35) or weekly low-dose schedules (36–40).

The cardiodepressant effects reported here occur when high DOX concentrations were used, and this fact could raise some doubts about its clinical relevance. The DOX concentrations achievable under different clinical circumstances have been both calculated (28,41) and directly measured (42,43). Lowe and Smallwood (28) have predicted a 5.5×10^{-6} M DOX plasma level 1 minute after a 60-mg/m^2 iv push, given over 5 minutes. Based on a two-compartment pharmacokinetic model, Wilkinson (41) predicted central compartment peak levels $> 10^{-5}$ M after a 60-mg/m^2 iv push dose. Furthermore, Piazza et al (42) measured DOX plasma levels in patients with solid tumors receiving 30 mg/m^2 of DOX iv. Peak plasma levels were $\geq 1.7 \times 10^{-5}$ M. Similar results were reported by

TABLE 5.—Effects of 60-min incubation with DOX on the chronotropic response to noradrenaline and histamine in guinea pig atria ($\bar{x}$ ± SEM)

Experimental group	Maximal chronotropic response (beats/min) to—	
	Noradrenaline	Histamine
Control	111.0 ± 6.1	109.5 ± 8.1
3×10^{-6} M DOX	107.0 ± 2.0	111.6 ± 4.4
10^{-5} M DOX	83.8 ± 9.5*	89.3 ± 9.0*
10^{-4} M DOX	67.5 ± 7.2†	62.3 ± 8.9†

*$P < 0.05$.
†$P < 0.01$.

Lee et al (43) in patients treated with 30 mg/m^2 of DOX iv over 5 minutes. It is conceivable that a 60-mg/m^2 iv push dose could achieve even higher peak plasma levels.

In conclusion, the results presented in this paper are compatible with the idea of an acute nonspecific interaction of DOX with beta-adrenergic and histaminic cardiac receptors. Since DOX could be cytotoxic even without entering the cell (44,45) and has shown the capability of modifying membrane fluidity (46), interaction with cell surface receptors could possibly play a role in DOX cardiotoxic mechanism. This interpretation does not exclude other cardiotoxic actions operating at membrane level or at intracellular sites (47–49).

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Analysis of "Early" Thymidine/Inosine Protection as an Adjunct to Methotrexate Therapy[1,2]

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The feasibility of "early" thymidine and inosine protection of methotrexate (MTX) toxicity is evaluated in this paper. This approach is based on the proposition that the most vulnerable period for susceptible host cells to MTX toxicity is an interval following a pulse of MTX when the MTX monoglutamate level is high. In contrast, tumor cells that accumulate active MTX polyglutamyl derivatives are exposed to the cytotoxic effects of MTX, not only when the monoglutamate levels are high, but also over the much longer interval during which polyglutamyl derivatives are retained within these cells. The protecting agents employed, thymidine and inosine, circumvent the antipurine and antipyrimidine effects of MTX, but neither agent inhibits the transport or the polyglutamylation of MTX. Nucleoside protection given over 6 hours after MTX markedly decreased toxicity to normal BDF_1 mice at MTX doses up to 150 mg/kg/day for 3 consecutive days. The LD10 for unprotected mice was 14 mg/kg/day, while the LD10 for the protected mice was 114 mg/kg/day, a greater than eightfold increase in the drug dose delivered. MTX with early nucleoside protection produced a 50% increase in median life span in tumor-bearing mice at doses of drug that were toxic to unprotected mice. Flow cytometric analyses indicated that consecutive daily pulses of MTX with early nucleoside protection alter the cell cycle distribution. By 24 hours after the last of three daily pulses of MTX, the G_1 component was maximal (80% of the cell population), with < 2% of the cells in G_2M and 17% of the cells in S phase. This distribution persisted for 24 hours, after which a normal pattern emerged, a process that was accelerated by neither thymidine/inosine nor leucovorin. These studies support the concept that toxicity to the host is initiated largely in the interval shortly after MTX administration. The data indicate that early nucleoside protection permits the repetitive administration of much higher doses of MTX than can be given with MTX alone. This approach may be useful not only in the treatment of human neoplasms but in the treatment of other disorders, such as rheumatoid arthritis, psoriatic arthritis, or psoriasis. Finally, this paper considers the potential advantages of early thymidine/inosine protection in comparison to leucovorin rescue with the administration of low or moderate doses of MTX. [Cancer Treat Rep 69:867–874, 1985]

The formation of methotrexate (MTX) polyglutamyl derivatives within tumor cells is a critical determinant of drug cytotoxicity (1). These derivatives of MTX have a high affinity for dihydrofolate reductase, and, because they are retained within cells (unlike intracellular monoglutamate which falls rapidly as the extracellular MTX level falls), they produce prolonged suppression of thymidylate synthesis and cell growth (2–4). Recently, polyglutamylation of MTX has been implicated as an important determinant of selectivity as well (5,6). Hence, accumulation of polyglutamyl derivatives of MTX in small intestinal cells in vivo is negligible in comparison to accumulation in murine tumors (5–8), and only low levels of MTX polyglutamates have been detected in hetergeneous popu-

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lations of bone marrow cells in vivo (7) and in granulocytic progenitor cell preparations selected from mouse bone marrow in vitro (9). Further, virtually no MTX polyglutamyl derivatives were formed upon exposure of granulocytic progenitor cell colonies to the drug in vitro (6).

Of particular interest is a comparison of the time course of accumulation of the monoglutamyl and polyglutamyl derivatives of MTX in tumor versus intestinal epithelial cells in vivo (5). Following sc administration of 12 mg/kg of MTX, > 90% of the monoglutamate exits small intestinal cells within 6-8 hours with negligible formation of polyglutamyl derivatives. In tumor cells, the peak monoglutamate level and its decline are similar to small intestinal cells. However, substantial polyglutamyl derivatives accumulate over the interval during which free intracellular drug is present; these derivatives are then retained in the intracellular compartment at levels in excess of the dihydrofolate reductase binding capacity over the next 24 hours (5).

These observations suggest that the major toxicity to intestinal cells may be initiated immediately after drug administration when appreciable levels of intracellular monoglutamate are present. However, the tumoricidal effect is due not only to this initial exposure to the monoglutamate but also to the much longer interval of exposure to the polyglutamyl derivatives. Thus, if the host could be protected during the initial interval of exposure to the monoglutamate while allowing polyglutamylation to proceed in the tumor, tumoricidal effects should be expressed following cessation of protection (when free monoglutamate levels in susceptible host and tumor cells are low but polyglutamyl derivatives persist in tumor cells) and exert a selective pharmacologic effect on the tumor alone.

The feasibility of early protection is evaluated in this paper by the use of thymidine/inosine protection over a short interval following administration of a pulse of MTX to normal and L1210 leukemia-bearing BDF_1 mice. These nucleosides completely prevent the antipurine and antipyrimidine effects of MTX by supplying the end products of tetrahydrofolate-dependent reactions (3,4,10) without interfering with the accumulation of MTX polyglutamyl derivatives within cells (4), a process which is inhibited by the simultaneous administration of MTX and leucovorin (11-13).

The experimental approach was designed to assess the toxicity and chemotherapeutic efficacy of this approach and to explore perturbations of the L1210 leukemia cell cycle, as determined by flow cytometry, when tumor-bearing mice receive daily pulses of MTX with "early" protection.

MATERIALS AND METHODS

MTX and thymidine were supplied by the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Inosine was obtained from the Sigma Chemical Co, St. Louis, MO. MTX was dissolved in water at pH 7-7.4 to obtain a stock solution of 20 mg/ml. For each experiment, a portion of the stock solution was diluted with water so that the correct dosage was delivered in a volume of 0.2 ml. Thymidine and inosine were dissolved together in water to a concentration of 100 mg/ml for each constituent by gentle warming in a water bath. Appropriate dilution of the stock solution in water was made in each experiment to deliver the thymidine/inosine dose in 0.1 ml. All solutions were refrigerated prior to use and were protected from light.

Studies were conducted with BDF_1 mice weighing 18-30 g. Standard laboratory mouse chow and water were provided ad libitum. L1210 leukemia cells were maintained in DBA/2 mice by weekly serial intraperitoneal transfer. Transplantation into BDF_1 mice was made on Day 7 following inoculation of the DBA/2 mice. In the survival studies, 2×10^5 L1210 cells were injected into BDF_1 mice on Day 0. MTX injections were begun on Day 1. All injections were administered sc in the dorsal neck.

Pilot studies indicated that optimal protection with maximal chemotherapeutic efficacy was achieved when thymidine and inosine doses of 300 mg/kg were administered simultaneously with, and at 2, 4, and 6 hours following, 100 mg/kg/day of MTX for 3 consecutive days. Less frequent administration of thymidine and inosine resulted in increased toxicity, while longer intervals of protection have been shown to diminish chemotherapeutic efficacy in tumor-bearing mice (14). The protection regimen employed in these studies utilized this dose and schedule of thymidine and inosine. Each experiment was performed with and without early protection at MTX doses of 12.5, 25, 50, 75, 100, and 150 mg/kg/day. Each experimental group consisted of four mice, and most experiments were performed in triplicate.

In the flow cytometry experiments, 10^4 L1210 leukemia cells were transplanted intraperitoneally into BDF_1 mice on Day 0. To accommodate the tumor kill and allow enough surviving cells to be analyzed by flow cytometry, MTX and thymidine/inosine injections were begun on Day 7. The mice were killed at the indicated times by cervical dislocation. The outer coat of the abdomen was removed and 1-2 ml of ice-cold 0.85% saline was injected ip. Cells were then aspirated from the intraperitoneal cavity, counted, and diluted with saline to approximately 2×10^6/ml, and were then stained using propidium iodide according to the method of Collins et al (15). All reagents used were obtained from Sigma Chemical Co, St. Louis, MO. The final concentration of the cells was approximately 10^6/ml. Stained cells were analyzed with an Epics V flow cytometer or a TPS-1 flow cytometer (Coulter Electronics, Inc, Hialeah, FL). The proportion of

cells in G_1, S, and G_2M was determined on a Tektronix 4051 mini-computer (Tektronix, Inc, Beaverton, OR) according to the method of Collins et al (15).

RESULTS

Effects of Early Protection on Host Toxicity

Figure 1 shows the results of a series of experiments in which mice were injected with MTX for 3 consecutive days at doses of 12.5–150 mg/kg/day with and without protection. In the unprotected mice, there is a linear relationship between MTX dose and percent of toxic deaths over the range of 12.5–100 mg/kg/day. At doses of ⩾ 100 mg/kg/day, all unprotected mice died. In contrast, there were no deaths at doses of ⩽ 75 mg/kg/day in protected mice. Toxic deaths appeared at higher doses, but this was minor in comparison to the unprotected mice, even at a MTX dose of 150 mg/kg/day. The LD10 for the unprotected mice was approximately 14 mg/kg/day (total dose, 42 mg/kg), while in the protected mice, the LD10 increased to 114 mg/kg/day (total dose, 342 mg/kg), a greater than eightfold increase in drug dose at comparable toxicity. Mantel and Haenszel's chi-square test was utilized to compare the proportion of surviving mice in the protected versus unprotected groups (16). This yields a chi-square value of 71 with a corresponding P value of 0.001, indicating that early protection significantly reduces drug-related mortality.

Effects of Early Protection on Chemotherapeutic Efficacy

Tumor-bearing mice (fig 2) were treated with three consecutive daily pulses of MTX at 12.5–150 mg/kg/day. There were three groups of mice, each inoculated with 2×10^5 L1210 leukemia cells. Group A received no treatment, Group B was treated with MTX alone, and Group C was treated with MTX and protection. The control mice (Group A not shown) had a median survival of 9 days. The unprotected mice (Group B) had a maximum median survival of 12.8 days at the lowest dose of 12.5 mg/kg/day, but survival declined precipitously with increasing doses of MTX because of increasing toxicity. In the protected mice (Group C), the maximum median survival of 13.5 days was comparable to the best survival with MTX alone, but an eightfold increase in dose (100 mg/kg/day) could be delivered. At MTX doses of ⩾ 25 mg/kg/day, 13 of 15 protected mice had longer survivals than mice treated with MTX alone. This difference is significant to a P value of 0.003.[6] Hence, at a MTX dose > 12.5 mg/kg/day for 3 days, protected mice survived longer than either control mice or mice treated with MTX alone.

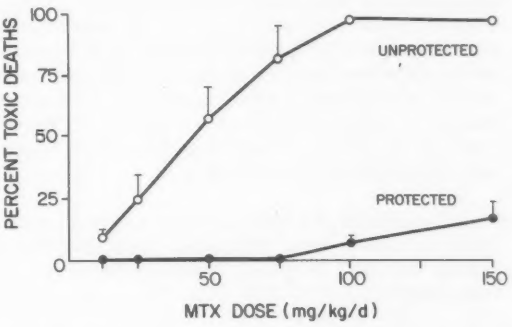

FIGURE 1.—Comparison of the toxicity of MTX administered for 3 consecutive days with and without early thymidine/inosine protection to BDF_1 mice. Data are the percent of toxic deaths (+ SE) based upon 3–4 separate experiments, with 4 mice/dose for protected and unprotected animals.

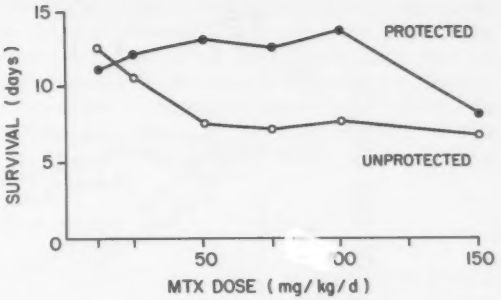

FIGURE 2.—Comparison of the chemotherapeutic efficacy of MTX with and without early thymidine/inosine protection. The indicated MTX dose was given daily for 3 consecutive days. The median of the median survivals for each dose from 3 separate experiments is plotted. Untreated tumor-bearing mice had a median survival of 9 days.

Effect of Repetitive Cycles of Multiple Daily Pulses of MTX

Figure 3 shows the change in weight of tumor-free mice produced by three repetitive cycles of treatment. Each cycle consisted of pulses of MTX at 50 or 75 mg/kg/day for 3 consecutive days with and without early protection followed by 3 days without treatment. Unprotected mice showed a continuous weight drop, with all mice dead by Day 16 (75 mg/kg/day) or Day 18 (50 mg/kg/day). At the lower dose of MTX (50 mg/kg/day), with protection, there was a modest weight loss which returned to within 0.5% of the baseline weight by the start of the second cycle and to within 5% of the baseline weight by the start of the third cycle. However, after the third cycle, there was a slow continuous weight loss resulting in the death of two

[6]Chance alone would predict that only one-half of the protected mice should survive longer than the corresponding unprotected mice. The probability that 13 of 15 mice would survive longer is equal to 0.003.

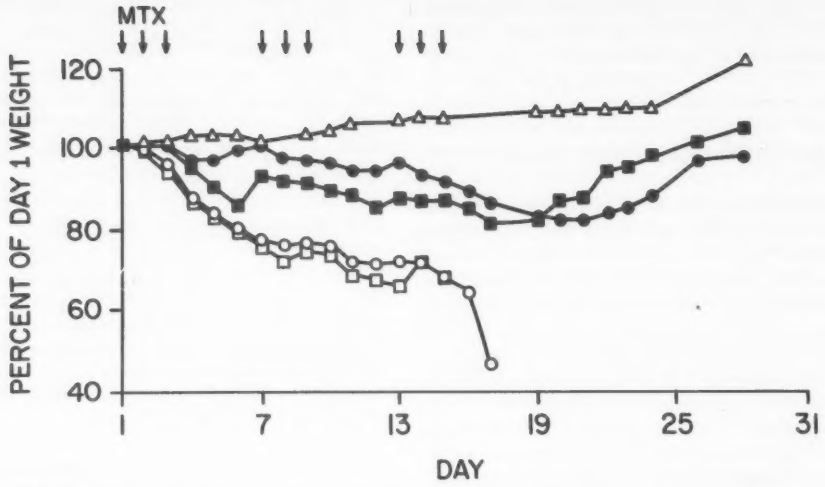

FIGURE 3.—Effect of repetitive cycles of MTX administered daily for 3 consecutive days with and without early thymidine/inosine protection on the weight of tumor-free BDF_1 mice. Day 1 weight was normalized to 100% for each group. For each dose of MTX, there were 8 mice in each protected group and 16 mice/group of unprotected mice. Four mice were in the control group (△). At 75 mg/kg/day of MTX, all unprotected mice were dead by Day 16 (□). At 50 mg/kg/day of MTX, all unprotected mice were dead by Day 18 (○). There was one death each on Days 24 and 26 at the 50-mg/kg/day MTX dose with early protection (●) and one death each on Days 20 and 22 at the 75-mg/kg/day MTX dose with early protection (■). The arrows indicate the days when MTX was administered.

of eight mice, following which the weights of the surviving mice returned to within 2% of baseline by Day 28. At the 75-mg/kg/day MTX dose, with protection, the 3-day interval was insufficient to allow recovery to the baseline weight before the start of the next cycle. This dose also resulted in the death of two of eight mice after the third cycle, with the surviving mice at the baseline weight by Day 28.

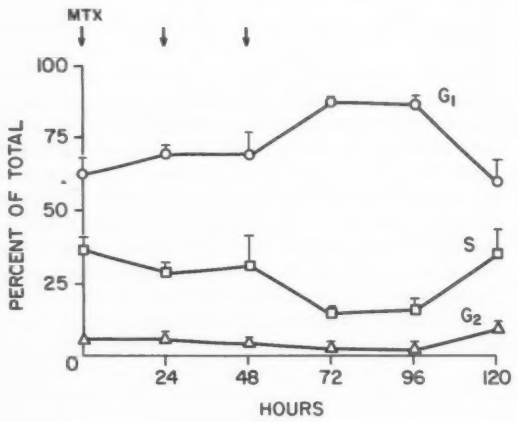

FIGURE 4.—Cell cycle distribution as a function of time after a fixed dose of MTX (50 mg/kg/day) was given at the indicated times (arrows) with early thymidine/inosine protection.

Because of the lesser toxicity of the 50-mg/kg/day MTX dose, this regimen was evaluated in tumor-bearing mice under conditions in which the second cycle was begun 3, 4, or 5 days after the third daily dose of MTX. There was no decline in median survival when a second cycle of MTX with early protection was given, and median survival appears to be comparable with a 3–5-day interval between cycles. Hence, a second cycle of MTX therapy with early protection can be administered without increased toxicity to tumor-bearing mice. Initial studies suggest that a second cycle of treatment results in improved efficacy when compared to a single cycle. Hence, when animals were given a second cycle of three consecutive daily doses of MTX (50 mg/kg/day) 3, 4, or 5 days after the end of the first cycle at this dose, the median survival of animals was 17 days (16, 16, 17, 17), 16.5 days (16, 16, 17, 19), and 16.5 days (16, 16, 17, 17), respectively, in comparison with 13 days for animals treated with one cycle alone (13, 13, 13, 13).

Flow Cytometric Analysis of L1210 Leukemia Cells Obtained From Mice Following Repetitive Daily Dosing With MTX With Early Thymidine/Inosine Protection

Flow cytometric analyses were employed to assess the effects of this therapeutic approach on cell cycle distribution. Figure 4 shows the time course of changes in the cell cycle distribution of L1210 leukemia cells as mice were pulsed with 50 mg/kg/day of MTX with protection for 3

consecutive days. There was little change in cell cycle distribution over the initial 48 hours. However, by 72 hours the G_1 component was maximal, approaching 80% of the cell population, with 2% of the cells in G_2M, and S-phase cells at a minimum of 17%. This cellular distribution persisted for the next 24 hours, even if thymidine/inosine was administered every 2 hours from 72–96 hours or when a single dose of leucovorin (400 mg/kg) was administered at 72 hours (not shown). Beyond 96 hours, a normal pattern of distribution of cells began to re-emerge spontaneously.

Further studies analyzed the pattern of distribution of cells at 72 hours as a function of the MTX dose (24 hours after the last of three consecutive daily pulses of MTX with early protection). The percentage of cells in G_2M shows a continuous fall as the dose of MTX is increased, reaching a minimum level at 20 mg/kg/day of MTX (fig 5). Concomitant with the fall in G_2M cells, the percentage of cells in S phase increases to nearly twice the baseline level at a MTX dose of 10 mg/kg/day and then falls as the MTX dose is increased. The percentage of cells in G_1 decreases to a nadir at the 10-mg/kg/day MTX dose and then increases. Figure 6 depicts representative flow cytograms of the perturbations induced by pulsing for 3 consecutive days with either 10 or 80 mg/kg/day of MTX with early protection as compared to control cells in log growth.

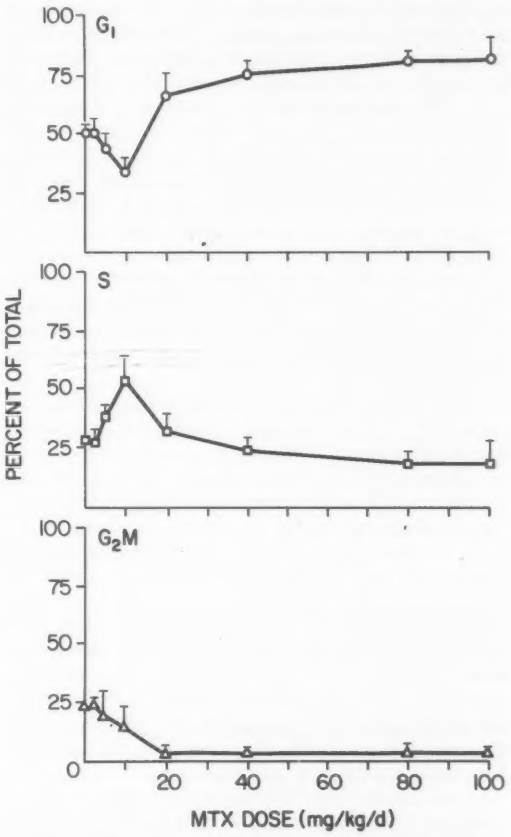

FIGURE 5.—Effect of MTX given daily for 3 days with early thymidine/inosine protection on cell cycle distribution. The data reflect analyses done 24 hrs after the last of the 3 pulses of MTX.

DISCUSSION

By blocking the synthesis of tetrahydrofolate from dihydrofolate, MTX prevents the transfer of one-carbon fragments necessary for purine and pyrimidine synthesis. Simultaneous administration of thymidine and a purine protects normal mice from methotrexate toxicity (14). Thymidine co-administered with MTX enhances the survival of tumor-bearing mice, an effect that is abolished when purine levels are raised, either by administration of allopurinol (17) or simultaneous administration of inosine (14). In humans, thymidine alone provides complete protection from otherwise lethal doses of MTX (18,19). The combination of thymidine protection and/or

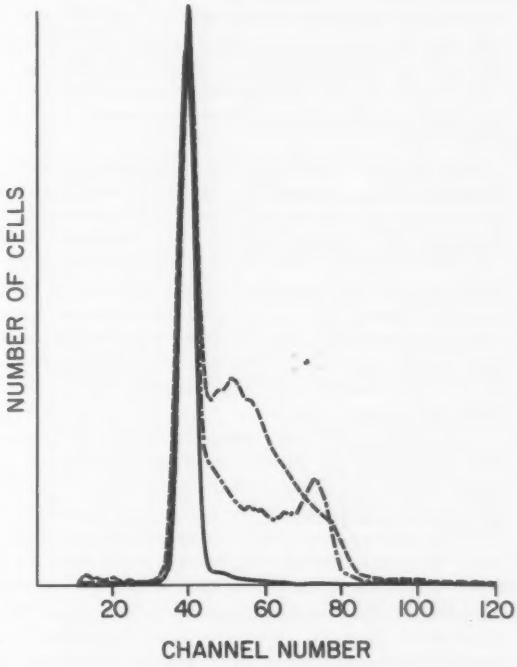

FIGURE 6.—Representative flow cytograms showing L1210 leukemia cells in log-phase growth (—·—), cells obtained from mice exposed to 80 mg/kg/day of MTX for 3 consecutive days with early protection (———), or 10 mg/kg/day of MTX for 3 consecutive days with early protection (— — —). The plot normalizes the G_1 peak to Channel 40.

rescue with high-dose MTX has been utilized in an attempt to enhance the chemotherapeutic efficacy of MTX. The experimental rationale in these studies has not been well-defined, aside from the suggestion that human bone marrow, with its higher purine concentration (20), might be protected by an infusion of thymidine alone, while tumor cells, which depend upon de novo purine synthesis and are exposed to lower plasma purine concentrations, would presumably undergo a purineless death. A variety of approaches have been employed. MTX has been infused for up to 72 hours with concurrent or prolonged thymidine administration (18,19). Most recently, MTX has been infused in patients with head and neck neoplasms with simultaneous high- or low-dose thymidine protection (21). None of these studies have demonstrated improved efficacy for MTX, but none have been designed to exploit known differences in the cellular pharmacology of MTX in tumor versus susceptible host tissues.

The approach that was utilized in this study is based on the difference in the accumulation of MTX polyglutamyl derivatives in tumor versus susceptible host tissues, ie, intestinal mucosal cells and bone marrow cells (5-7,9), and the proposition that the most vulnerable period for cells which do not produce appreciable levels of MTX polyglutamates is immediately after a pulse of MTX when the monoglutamate level is high. In contrast, tumor cells are exposed to the cytotoxic effects of MTX, not only over the interval during which the monoglutamate level is high, but also over the much longer interval during which polyglutamyl derivatives are present within these cells.

These data establish the validity of the concept of early thymidine/inosine protection. Effective protection of normal mice was demonstrated with MTX doses to 100 mg/kg/day for 3 consecutive days, with an increase in the LD10 for MTX by a factor of 8 as compared to unprotected animals. The protection regimen consisted of four pulses of thymidine/inosine over 6 hours. Because of the rapid clearance of nucleosides, effective protection probably ceases 1-3 hours after the last thymidine/diglycoaldehyde dose, thereby providing protection for a total of 6-8 hours (23,24).

Three consecutive daily doses of MTX with early protection enhanced the survival of tumor-bearing mice in comparison to mice treated without protection at MTX doses > 12.5 mg/kg/day. While this is no more effective than the optimal dose of MTX given without protection over the same 3-day interval, this approach permits an eightfold increase in the dose of MTX without an increase in toxicity. Furthermore, since optimization of the thymidine/inosine dose and schedule was not evaluated for each MTX dose, changes in the dosage and/or duration of the window of protection might further increase chemotherapeutic efficacy.

This approach might be of particular importance in the treatment of tumors with impaired transport of MTX, increased levels of dihydrofolate reductase, or enzyme with reduced affinity for the drug where higher doses of MTX would be necessary to produce a pharmacologic effect, but normally could not be administered without unacceptable toxicity (25-32). This approach may also have utility in the treatment of nonmalignant diseases such as rheumatoid arthritis, psoriatic arthritis, or psoriasis, where low-dose pulses of MTX have been used with moderate success (33-35). If the effector cells in these diseases form polyglutamyl derivatives of MTX, then this method might allow much larger doses of MTX to be given without an increase in toxicity and with an enhanced therapeutic effect.

A second cycle of administration of MTX with each cycle consisting of three daily pulses of 50 mg/kg/day of MTX plus early thymidine/inosine protection followed by a 4-day rest period permits an increase in the total dose of MTX delivered without a comparable increase in toxicity. This may improve survival beyond that achieved with a single cycle, but cannot be curative in this L1210 leukemia cell system. Hence, if a 4-day interval is required between treatment cycles to avoid excessive toxicity (fig 3), each treatment would extend over a 7-day period. Based on an L1210 leukemia cell doubling time of 12 hours (36), there would be a 3-log increase in tumor cells over this interval, while each cycle would result in only an approximately 2-log kill. Therefore, each cycle would allow a net increment of at least 1 log of tumor cells, and delay, but not prevent, death. This approach might, however, be curative in tumors with more inherent sensitivity to MTX. This is similar to the limitation encountered with high-dose MTX with leucovorin rescue, ie, achievement of a 2-log kill with MTX but sufficient toxicity to prevent the drug from being administered more often than weekly, and, hence, a 3-log growth in tumor cells over this interval (17).

A possible consequence of repetitive daily administration of MTX is the development of cell synchrony and the increasing buildup of MTX polyglutamyl derivatives with each dose of drug. Following cessation of protection, intracellular MTX polyglutamyl derivatives that have accumulated should result in the death of tumor cells in S phase or accumulation of other tumor cells at the G_1-S interface or in early S phase. Upon administration of the subsequent dose of MTX along with protecting nucleosides, cells should proceed with semiconservative DNA synthesis in S phase. However, these cells are "caught" in what is their most vulnerable phase when the protecting nucleosides are withdrawn and cleared 6-8 hours later and intracellular MTX polyglutamate levels are high. This sequence of events should repeat itself with each daily pulse of MTX with protection.

As the number of MTX pulses and the drug dose were increased, a progressive shift of cells into G_1 was found by flow cytometric analyses performed at 24-hour intervals. Furthermore, after three daily pulses of MTX, cells

re-entered the cell cycle only slowly and at a rate that was accelerated by neither thymidine/inosine nor leucovorin. It might be possible to exploit this homogeneity of the cellular population in G_1 that persists 24–48 hours after the last dose of MTX by the addition of an agent that has activity in this cell cycle phase, such as cisplatin (37), at a dose sufficient to achieve a 2–3-log cell kill but with minimal added toxicity. Other approaches are possible. For instance, when cells begin to replicate following cessation of MTX, MTX could be restarted, as was done with the second cycle of therapy, or another S phase-specific agent such as cytarabine could be administered. Of interest was the observation that 72 hours after the start of a low-dose cycle of MTX (10 mg/kg/day), there was a buildup of cells in S phase (fig 5); this might be an optimal time to administer an S phase-specific agent.

Early nucleoside protection could provide a potential advantage for chemotherapy programs with frequent MTX dosing as compared to leucovorin rescue. Hence, when leucovorin is administered following MTX, selectivity is enhanced as host cells are rescued and tumor cells are killed (22). However, leucovorin administered after MTX may decrease the cytotoxicity to tumor cells of the next dose of MTX, because treatment of tumor cells with leucovorin impairs the polyglutamylation of MTX (11,38). Furthermore, the loading of cells with tetrahydrofolate cofactors could also result in high levels of dihydrofolate in the presence of MTX, resulting in increased competition with the antifolate at the level of dihydrofolate reductase. This could represent a biochemical basis for a self-limiting effect of repetitive cycles of MTX with leucovorin rescue, particularly when the interval between the cycles is brief. Early nucleoside protection, on the other hand, should preserve selectivity with repetitive administration because of the rapid consumption of intracellular and extracellular nucleosides. This leaves tumor cells in an essentially unchanged state, able to metabolize MTX normally with an unaltered tetrahydrofolate cofactor distribution, at the time the next dose of MTX is administered.

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Effect of Cisplatin on Organic Ion Transport in Membrane Vesicles From Rat Kidney Cortex[1]

Patricia D. Williams* and G. H. Hottendorf[2]

Purified renal membrane vesicles were utilized to gain indirect information regarding the renal handling of cisplatin. The effects of cisplatin on prototypical organic anion (*p*-aminohippurate, PAH) and cation (N^1-methylnicotinamide; tetraethylammonium, TEA) transport in brush border and basolateral membrane vesicles prepared from rat kidney cortex were observed. While cisplatin inhibited organic cation transport (N^1-methylnicotinamide; TEA) in brush border and basolateral membranes, no interaction with the organic anion (*p*-aminohippurate) system was observed. Kinetic analyses revealed that cisplatin is a competitive inhibitor of TEA transport in brush border membranes with a k_i of 0.12 mM. While the relationship between organic cation transport inhibition and cisplatin nephrotoxicity is unknown, it may suggest that the cisplatin complex itself is transported into the kidney by the organic cation system. The reported effect of the organic anion, probenecid, on the renal handling of cisplatin is discussed in light of these results. [Cancer Treat Rep 69:875–880, 1985]

Cisplatin is a heavy metal complex containing a central platinum atom surrounded by two ammonia molecules and chloride atoms arranged in the *cis* position. Cisplatin is an effective cancer chemotherapeutic agent, utilized primarily in the treatment of metastatic testicular and ovarian tumors (1,2). The clinical use of cisplatin is limited chiefly by dose-related and cumulative nephrotoxicity (3,4). Renal insufficiency has been reported to occur in 28%–36% of the patients treated with a single dose of cisplatin (5). This nephrotoxicity is characterized by destruction of proximal tubular cells situated in the corticomedullary section of the kidney (6–8). While cisplatin is known to accumulate in the kidney (9,10), the mechanism of its renal cellular accumulation is unknown. It has been demonstrated that the organic anion, probenecid, increases the renal clearance of cisplatin, which is consistent with inhibition of tubular reabsorption of cisplatin (11). However, it is not known whether probenecid is affecting the transport of cisplatin itself or a cisplatin reaction product or metabolite.

It was the purpose of this study to gain indirect information regarding the renal handling of the cisplatin complex itself by observing effects of intact cisplatin on renal organic ion transport. Renal transport systems that have evolved to translocate endogenous organic ions may be utilized to accumulate various drugs and/or other exogenous chemicals which act as substrates for these systems (12,13). Thus, information regarding renal transport mechanisms for xenobiotics can be achieved by observing effects of chemicals on the transport of endogenous substances such as organic acids and bases (14,15). Competition for transport of such substances provides indirect evidence of the transport systems utilized to translocate xenobiotics.

The renal tubular cell is anatomically and functionally differentiated into the brush border (luminal) and basolateral (blood surfaces) poles. Transport processes operating at the brush border and basolateral membranes are key events in the renal cellular accumulation and elimination of endogenous as well as foreign substances. Since processes occurring simultaneously at these two membranes are not directly observable by in vivo measurements, the use of purified renal membrane vesicles was employed to examine separately the brush border and basolateral membrane transport effects of cisplatin. Isolated kidney membrane vesicles have been utilized to study renal transport mechanisms of a variety of substances including organic and inorganic ions (16,17), sug-

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ars (18), amino acids (19), and drugs (20,21).

MATERIALS AND METHODS

Experimental Animals

Male Sprague-Dawley rats (200-250 g) were utilized for these experiments. The rats were killed by cervical dislocation. Following cervical dislocation, the kidneys were immediately removed and placed in ice-cold saline. Cortical tissue was separated from the medulla, weighed, and homogenized in 10 volumes (wt/vol) of 0.25 M sucrose using a teflon-glass homogenizer.

Membrane Isolation and Transport Assays

Brush border and basolateral membrane vesicles were prepared by a modification of the method of Kinsella et al (22) and Williams et al (23). Basolateral membranes were collected on a discontinuous sucrose gradient composed of 8% and 38.7% sucrose. Purity of the two membranes relative to the starting homogenate was determined by measuring the enhancement of marker enzymes associated with the membranes. Membranes were suspended in a buffer consisting of 100 mM mannitol and 20 mM HEPES, pH 7.4, with KOH.

Transport studies were performed at 25°C in the buffer described above. Transport characteristics of cisplatin were studied in an indirect manner by observing the effects of cisplatin on the initial rates of membrane transport of a prototypical organic anion (*p*-aminohippurate, PAH) and organic cations (N^1-methylnicotinamide, NMN; tetraethylammonium, TEA). Transport assays were performed by incubating membrane vesicles with radiolabeled PAH, NMN, and TEA in the presence and absence of cisplatin for 30 seconds. The specificity of PAH, NMN, and TEA transport was determined by incubation with respective inhibitors of organic ion transport, probenecid (anion), and mepiperphenidol (cation). The reaction was initiated by the addition of membrane protein (100 μg) and was stopped by the addition of excess cold transport buffer. The membranes were collected on 0.45-μm Millipore filters under vacuum and transport was quantitated by counting radioactivity by liquid scintillation spectroscopy.

Preparation of Cisplatin Solutions and Compatibility With Transport Substrates

Unreacted cisplatin was prepared by reconstituting commercially prepared cisplatin in Milli-Q purified water (Millipore Corporation). The commercial vials of cisplatin contained 90 mg of sodium chloride and 10 mg of cisplatin/vial. Cisplatin solutions were reconstituted to a concentration of 5 mM cisplatin and 232 mM sodium chloride. These solutions contained unreacted cisplatin since it has been demonstrated that chloride > 100 mM prevents the formulation of cisplatin hydrolysis products (24). The integrity of cisplatin added to the transport buffer was confirmed by high-performance liquid chromatography analyses.

Statistics

Data are presented with standard errors. Statistical significance was determined by Dunn's multiple comparison method (25). Kinetic plots were prepared by linear regression analyses of the data.

Protein and Enzyme Assays

Protein was determined by the Lowry method using bovine serum albumin as the standard (26). Na^+, K^+-ATPase was measured by a linked-enzyme assay in which the oxidation of NADH was continuously monitored at 340 nm in the presence and absence of 1 mM ouabain (27). Alkaline phosphatase was measured at 420 nm by the *p*-nitrophenyl phosphate method (28), and succinic dehydrogenase was monitored at 600 nm by a method described by Earl and Korner (29).

Materials

Cisplatin utilized in this study was commercially available material (Platinol, Bristol-Myers Company, Syracuse, NY). ^{3}H-PAH was purchased from Amersham Radiochemicals and ^{3}H-TEA was purchased from New England Nuclear Corporation (Boston, MA). ^{3}H-NMN was custom-synthesized by ICN, Irvine, CA. Mepiperphenidol chloride (Darstine) was purchased from Merck, Sharp & Dohme Laboratories (West Point, PA). All other chemicals and reagents were purchased from Sigma Chemical Company.

RESULTS

Membrane Isolation and Purification

Brush border and basolateral renal membrane vesicles were isolated and purified as indicated by enhancement of marker enzyme activities relative to the starting material shown in table 1. Alkaline phosphatase, a brush border membrane marker, was enriched 11-fold over the starting homogenate, while the basolateral membrane marker, Na^+,K^+-ATPase, showed a 13-fold enrichment. Both membranes showed a lack of enrichment of the mitochondrial enzyme marker, succinic dehyrogenase.

Effects of Cisplatin on Organic Ion Transport

In measuring initial rates of PAH and TEA uptake into membrane vesicles, the effects of cisplatin are shown in

TABLE 1.—Specific activity* of marker enzymes in starting material and purified membranes of rat renal cortex†

Kidney fraction	Na^+,K^+-ATPase	Alkaline phosphatase	Succinic dehydrogenase
Homogenate	7.94 ± 1.44	20.22 ± 1.14	3.32 ± 0.13
Brush border membrane	12.86 ± 4.82	226.80 ± 12.20	0.50 ± 0.11
Basolateral membrane	102.00 ± 14.36	33.52 ± 1.00	1.42 ± 0.02

* Enzyme activity in units/mg of protein/hr.
†Values = mean ± SE for 3–5 preparations.

TABLE 2.—Effects of cisplatin on ^{3}H-PAH transport in brush border and basolateral membranes from rat renal cortex

Membrane fraction	Additions*	PAH transport†	% inhibition
Brush border	None	19.35 ± 2.3 (5)	—
	Probenecid	6.98 ± 1.4 (7)	64‡
	Cisplatin	21.87 ± 3.2 (6)	+13
Basolateral	None	45.37 ± 6.1 (4)	—
	Probenecid	5.78 ± 1.8 (5)	87‡
	Cisplatin	47.84 ± 1.9 (4)	+5

* Additions of probenecid (final concentration, 10 mM) and cisplatin (final concentration, 1 mM) were made to incubation mixture containing ^{3}H-PAH (50 μM) and membranes (100 μg) in transport buffer.
†Transport studies were performed at 25°C in 100 mM mannitol and 20 mM HEPES, pH 7.4. ^{3}H-PAH was present at a concentration of 50 μM. Reaction was initiated by addition of 100 μg of membrane protein and stopped after 30 secs by addition of excess cold transport buffer. Transport assays expressed in pmols of PAH uptake/mg of protein/min. Values = mean ± SE. Nos. in parentheses = No. of experiments.
‡Statistically significant, $P < 0.05$.

TABLE 3.—Effects of cisplatin on ^{3}H-TEA transport in brush border and basolateral membranes from rat renal cortex

Membrane fraction	Additions*	TEA transport†	% inhibition
Brush border	None	120.14 ± 5.8 (6)	—
	Mepiperphenidol	4.13 ± 1.8 (8)	97‡
	Cisplatin	40.01 ± 3.1 (6)	67‡
Basolateral	None	66.15 ± 8.2 (6)	—
	Mepiperphenidol	6.16 ± 1.9 (8)	91‡
	Cisplatin	32.39 ± 5.7 (6)	51‡

* Additions of mepiperphenidol (final concentration, 10 mM) and cisplatin (final concentration, 1 mM) were made to incubation mixture containing ^{3}H-TEA (50 μM) and membranes (100 μg) in transport buffer.
†Transport studies were performed at 25°C in 100 mM mannitol and 20 mM HEPES, pH 7.4. ^{3}H-TEA was present at a concentration of 50 μM. Reaction was initiated by addition of 100 μg of membrane protein and stopped after 30 secs by addition of excess cold transport buffer. Transport assays expressed in pmols of TEA uptake/mg of protein/min. Values = mean ± SE. Nos. in parentheses = No. of experiments.
‡Statistically significant, $P < 0.05$.

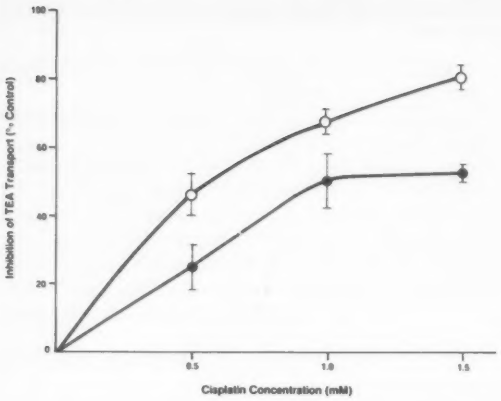

FIGURE 1.—Dose-response of cisplatin inhibition of ^{3}H-TEA transport in brush border (○) and basolateral (●) membrane vesicles from rat renal cortex. Initial velocity of 50 μM ^{3}H-TEA uptake was measured after incubating 100 μg of membrane protein with ^{3}H-TEA in the presence or absence of cisplatin for 30 secs. Transport buffer contained 100 mM mannitol and 20 mM HEPES, pH 7.4, with KOH. Each data point represents average of 6 experiments ± SE.

tables 2 and 3. As expected, probenecid and mepiperphenidol exhibited significant inhibition of organic anion and cation transport, respectively. Cisplatin significantly inhibited TEA transport in both membranes, while effects on PAH transport were not observed. The dose-response of TEA inhibition in brush border and basolateral membranes is shown in figure 1.

To probe the mechanism of TEA transport inhibition by cisplatin, the interaction of cisplatin and TEA transport was examined kinetically (figs 2 and 3). The linear regression plots were highly correlated with the original data (r values > 0.96; $P < 0.05$ for each line). Dixon plot analysis of the data revealed that while cisplatin competitively inhibited TEA transport in brush border membrane vesicles with a k_i of 0.12 mM, its interaction with TEA transport in basolateral membrane vesicles did not demonstrate a competitive mechanism. These observations are consistent with reports that the organic cation carrier in the brush border differs from the carrier in the basolateral membrane (16,30).

Further evidence that the cisplatin complex itself interacts with cationic transport systems in the kidney was obtained by observing the effects of cisplatin on membrane vesicle uptake of another prototypical organic cation, NMN. As shown in table 4, cisplatin significantly inhibited brush border and basolateral membrane uptake of this cation.

DISCUSSION

The results of this investigation demonstrate that cisplatin significantly inhibits cationic transport systems in brush border and basolateral membrane vesicles from rat kidney. Since unreacted cisplatin is a neutral species, the nature of the interaction may involve the nitrogen of the ammonia groups per se rather than a positive charge interaction. At the brush border, the interaction with TEA transport is competitive in nature and may therefore represent an avenue of renal cellular accumulation via tubular reabsorption of cisplatin. While the net direction (secretion or reabsorption) of cisplatin transport in the kidney is unresolved, tubular reabsorption of cisplatin has been reported (31–34). A recent report has cited the lack of interaction of the organic cation, NMN, on the renal handling of cisplatin (34). However, only total platinum recovery was measured. Thus, the behavior of the intact cisplatin complex was not monitored. Additionally, since the experiment was performed in vivo, the presence of numerous serum reaction products could likely mask any effects on cisplatin. The lack of interaction of cisplatin with the organic anion transport system described here suggests that the reported effects of probenecid on the renal handling of cisplatin in vivo may represent effects on cisplatin-serum reaction products rather than cisplatin itself, as suggested by Daley-Yates and McBrien (11). Since the role of cisplatin versus cisplatin reaction products in the renal pathogenesis of cisplatin is still unknown, the relative importance of renal cellular internalization via cationic or anionic transport systems in the development of nephrotoxicity is not known. However, the failure of probenecid to reduce cisplatin nephrotoxicity in man (35) coupled with the observation that protein-bound cisplatin is not nephrotoxic (36) suggests that the renal transport and accumulation of the cisplatin complex itself may be an important event in the pathogenesis.

In conclusion, these data demonstrate an interaction between cisplatin and cationic transport systems in brush border and basolateral renal membrane vesicles of the rat. These findings suggest that the cisplatin complex itself may be accumulated in renal tissue by a facilitated, carrier-mediated transport system which translocates organic cations. Since the plasma membrane represents the initial barrier between the cell and its environment, renal membrane transport is the primary event leading to renal accumulation and subsequent cellular damage due to nephrotoxins. Membrane vesicles provide a model which can be utilized to measure this primary interaction and also offer a mechanism to investigate strategies of preventing the process of cellular internalization and subsequent injury. Thus, the study of membrane transport mechanisms of cisplatin may reveal avenues of ameliorating the nephrotoxicity associated with this agent.

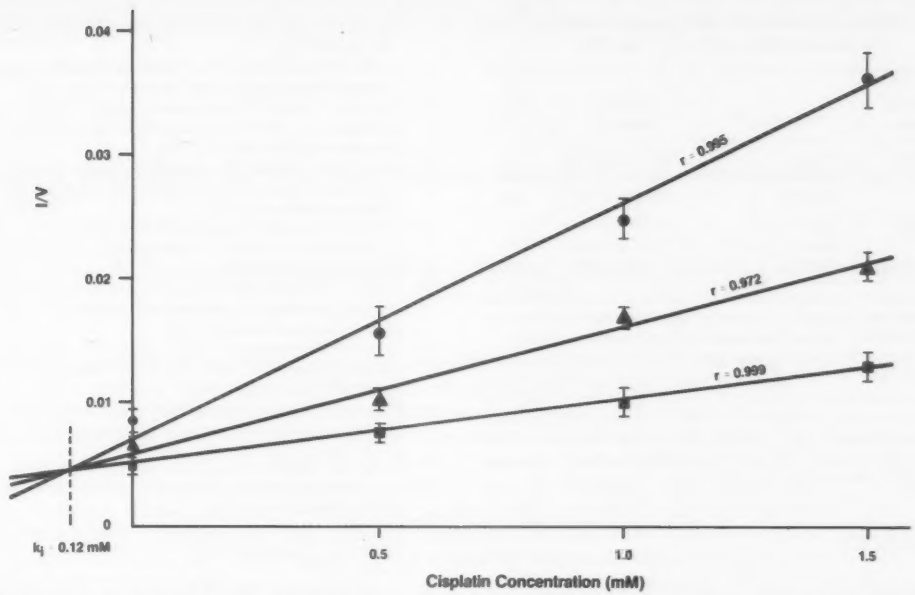

FIGURE 2.—Dixon plot of cisplatin effect on brush border membrane uptake of ^{3}H-TEA. Transport of 50 (●), 75 (▲), and 100 (■) μM ^{3}H-TEA is shown in the absence and presence of 0.5, 1.0, and 1.5 mM cisplatin. Each data point represents average of 6 experiments ± SE. Transport is expressed as I/V, where V = pmols of TEA transported/mg of protein/min. Correlation coefficients (r) are represented on each line.

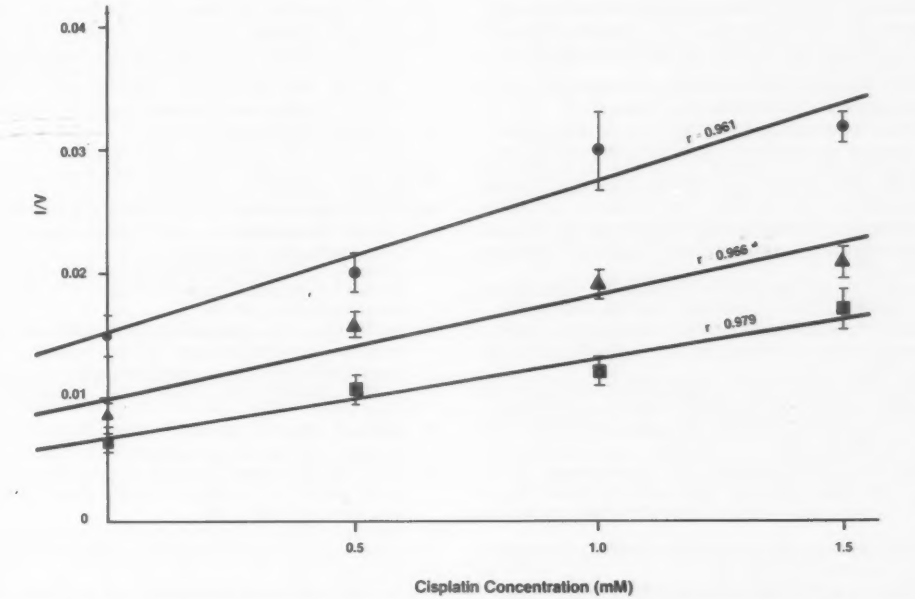

FIGURE 3.—Dixon plot of cisplatin effect on basolateral membrane uptake of ^{3}H-TEA. Transport of 50 (●), 75 (▲), and 100 (■) μM ^{3}H-TEA is shown in the absence and presence of 0.5, 1.0, and 1.5 mM cisplatin. Each data point represents average of 6 experiments ± SE. Transport is expressed as I/V, where V = pmols of TEA transported/mg of protein/min. Correlation coefficients (r) are represented on each line.

TABLE 4.—Effects of cisplatin on ^{3}H-NMN transport in brush border and basolateral membranes from rat renal cortex

Membrane fraction	Additions*	NMN transport†	% inhibition
Brush border	None	123.73 ± 12.3 (6)	—
	Mepiperphenidol	45.10 ± 5.7 (6)	64‡
	Cisplatin	76.83 ± 4.9 (6)	38‡
Basolateral	None	137.70 ± 11.0 (6)	—
	Mepiperphenidol	41.27 ± 2.8 (6)	70‡
	Cisplatin	73.27 ± 6.7 (6)	47‡

*Additions of mepiperphenidol (final concentration, 10 mM) and cisplatin (final concentration, 1 mM) were made to incubation mixture containing ^{3}H-NMN (50 μM) and membranes (100 μg) in transport buffer.

†Transport studies were performed at 25°C in 100 mM mannitol and 20 mM HEPES, pH 7.4. ^{3}H-NMN was present at a concentration of 50 μM. Reaction was initiated by addition of 100 μg of membrane protein and stopped after 30 secs by addition of excess cold transport buffer. Transport assays expressed in pmols of NMN uptake/mg of protein/min. Values = mean ± SE. Nos. in parentheses = No. of experiments.

‡Statistically significant, $P < 0.05$.

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High-Dose Cytarabine for the Treatment of Blastic Phase Chronic Myelogenous Leukemia[1,2]

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High-dose cytarabine chemotherapy regimens were given to 22 patients for the treatment of blastic phase chronic myelogenous leukemia. Bone marrow aplasia occurred in 21 of these patients; in one patient the marrow was not cleared of blasts. In five patients (26%), blastic phase promptly recurred. Eight patients (37%) died of infection or hemorrhage during the period of marrow aplasia before bone marrow recovery. Seven patients achieved complete remission and one achieved partial remission, but the duration of the remission was brief [median, 98 days (range, 52–345)]. One patient received consolidation therapy with an additional course of high-dose cytarabine and maintained remission for 345 days. These results suggest that alternative approaches to the treatment need to be explored. [Cancer Treat Rep 69:881–883, 1985]

Chronic myelogenous leukemia (CML) most frequently terminates after 3 years in an accelerated or blastic phase, which is generally resistant to therapy (1). Complete remissions, using a variety of chemotherapy regimens, are achieved in about 10%–40% of the patients during the blastic phase, but the durations of remission are brief (2–8). The most frequently used protocols employ vincristine and prednisone combinations and/or conventional-dose cytarabine with anthracycline antibiotics. The vincristine-prednisone regimens have been particularly useful in patients whose leukemic blasts are terminal deoxynucleotidyl transferase-positive. The successful use of high-dose cytarabine (HDARAC) for patients with refractory acute leukemia suggested its use in patients with blastic phase CML (9–11).

PATIENTS AND METHODS

Twenty-two patients with Philadelphia (Ph^1) chromosome-positive blastic phase CML were studied. The median age was 37 years (range, 23–69). During the chronic phase, all of the patients had received chemotherapy with hydroxyurea, busulfan, or both. The following criteria for the diagnosis of accelerated phase or blast crisis were used (2): peripheral blasts and progranulocytes > 20%; resistance to conventional treatment (as manifested by increasing anemia, thrombocytopenia, or wbc count); extramedullary infiltration with myeloblasts; progressive or new cytogenetic chromosomal changes; and increasing hepatomegaly or splenomegaly. Terminal deoxynucleotidyl transferase (TdT) was positive in five patients, all of whom had failed to respond to vincristine-prednisone chemotherapy. Eight patients were TdT-negative; nine were not tested. Five of the 22 patients had received conventional-dose cytarabine during the accelerated phase. The studies were approved by each institution's Human Investigation Committee and informed consent was obtained.

Cytarabine was prepared and administered as previously reported (9). Cytarabine (3 g/m^2) was given iv over 1 hour every 12 hours for four to six doses with L-asparaginase, etoposide, or daunorubicin (nine patients); for 12 doses alone (two); for 12 doses with daunorubicin (seven); or for 16 doses alone (one). Two patients received 4.5 g/m^2 iv over 1 hour every 12 hours for 12 doses. Patients received a variety of

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HDARAC regimens as part of phase I-II studies of HDARAC in acute leukemia. These phase I-II studies were initiated to determine the maximally tolerated therapy for HDARAC. One patient received another course of HDARAC (12 doses) 3 months after achieving complete remission; no maintenance therapy was given to the remaining patients.

The criteria for toxicity have been previously reported (9,12). Briefly, grade 0 was the absence of toxicity, grade 1 was mild organ dysfunction, grade 2 was moderate organ dysfunction requiring supportive care for < 2 weeks, grade 3 was severe organ impairment but reversible, and grade 4 was irreversible or fatal toxicity.

The criteria for response were as follows. Complete remission (CR) was defined as the complete clearance of blasts and progranulocytes from the peripheral blood with a normal hemogram, and < 5% blasts (or < 10% blasts and progranulocytes) in a normocellular bone marrow, with or without cytogenetic disappearance of the Ph^1 chromosome. A partial remission (PR) was defined the same as CR except the bone marrow was either hypocellular or exhibited granulocytic hyperplasia consistent with the chronic phase of CML. A leukemic response was defined as achieving marrow aplasia but patient death before marrow recovery. Persistent leukemia was defined as failure to clear the bone marrow of leukemic blasts. Recurrent leukemia was defined as the recovery of bone marrow in the accelerated or blastic phase. When all the responders relapsed, the blastic phase had reappeared. Thus, the duration of response was measured by the reappearance of blast phase.

RESULTS

Toxicity

Twenty-one patients completed the induction therapy; one patient died of hemorrhage after 4 days of HDARAC while pancytopenic. All of the 21 remaining patients experienced fever while pancytopenic; nine of these had documented, culture-proven infections. Granulocyte count recovery (polymorphonuclear neutrophil count > 500/μl) occurred a median of 34 days (range, 22-100) after starting therapy; platelet count recovery (> 20,000/μl untransfused) occurred a median of 27 days (range, 11-72) after beginning treatment. One patient experienced grade 3 hepatic toxicity (elevation of tests of liver function three to five times normal). Two patients experienced irreversible CNS toxicity with the highest dose of cytarabine and were reported previously (12). The remainder of the side effects (ocular, dermal, and gastrointestinal) associated with HDARAC were similar to those observed in previous studies (9-14).

Response

Twenty-one patients achieved marrow aplasia, including the patient who died after 4 days of therapy; one patient had persistent leukemia after completing therapy. Eight patients (36%) had a leukemia response, but died of hemorrhage or infection before recovery of bone marrow function a median of 27 days after initiating therapy (range, 5-64 days). Thirteen patients recovered bone marrow function: five (23%) had persistent blastic phase CML and eight achieved remission (seven CRs; one PR). The five patients with persistent blastic phase survived a median of 49 days after initiating therapy (range, 30-158). Ph^1 chromosome analysis was performed on six of the seven patients achieving CR, with three demonstrating an absence of chromosomal abnormality. Two of the patients with CR were TdT-positive, vincristine-prednisone treatment failures. The median duration of unmaintained CR was 98 days (range, 52-126). The patient with PR had an unmaintained remission of 90 days. The patient who received consolidation therapy with HDARAC had a remission duration of 345 days.

DISCUSSION

The results of our previous studies using HDARAC suggested that similar therapy for the blastic phase of CML might be successful (9-11). The 32% CR rate (seven responses among 22 patients) compares favorably with the reported CR rates ranging from 10% to 40% (2-6). Recent studies from several centers have demonstrated a similar but lower response rate using HDARAC, reporting three responses among 17 patients in aggregate (13-18). Interestingly, two of five patients in the present report, who were TdT-positive and had failed induction with vincristine-prednisone, achieved CR. This group of patients does not usually respond to therapy after failing vincristine-prednisone (3,8). Further, HDARAC was effective in producing aplasia in 21 of the patients; recurrent or persistent leukemia occurred in approximately one-quarter of the patients. The one case in which consolidation therapy was given may be instructive. This patient had a CR of nearly 1 year, and after relapse, a second brief CR was achieved using HDARAC. HDARAC for consolidation therapy in patients with acute nonlymphocytic leukemia has been shown to prolong remissions (19), and a similar method may be beneficial for responding patients.

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Continuous Infusion of Vinblastine for Advanced Hormone-Refractory Prostate Cancer[1]

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Thirty-nine patients with metastatic prostate cancer refractory to hormonal manipulation were treated with vinblastine by continuous infusion. The dose was 1.5 mg/m^2 daily for 5 days. A 21% response rate was obtained. Eight patients, two with visceral metastasis, one with nodal disease, and five with osseous metastasis achieved objective response. The median duration of response was 28 weeks. Myelosuppression was the major side effect: the median leukocyte count nadir was 2.8 × 10^3/ml and the median platelet count nadir was 184 × 10^3/ml. [Cancer Treat Rep 69:885–886, 1985]

Vinca alkaloids have not received extensive evaluation against metastatic prostate cancer. Yap et al (1) have documented a 40% response rate and good patient tolerance with vinblastine infusion for patients with advanced refractory adenocarcinoma of the breast. Antineoplastic agents effective in breast carcinoma are frequently active against prostatic carcinoma. We therefore initiated a phase II study of continuous-infusion vinblastine for patients with advanced hormone-refractory prostate cancer.

MATERIALS AND METHODS

Vinblastine infusion therapy was used in 39 patients with metastatic and hormone-refractory adenocarcinoma of the prostate. Eligible patients were those who failed prior chemotherapy or in whom doxorubicin was contraindicated. Patient characteristics are shown in table 1. Vinblastine (Velban, Eli Lilly and Company) was given by continuous iv infusion at a dose of 1.5 mg/m^2/day × 5 days. Courses were repeated every 3–4 weeks. Vinblastine was administered through a centrally placed iv line. Treatment was administered primarily on an outpatient basis.

Criteria for objective response, as previously defined (2), were as follows. (*a*) Subjective symptoms of the disease, when present, must improve. (*b*) For measurable metastases, the sum of the products of perpendicular diameters must be reduced by at least 50%. (*c*) Osseous metastasis must fulfill all of the following criteria: lytic lesions, if present, must recalcify; elevated levels of alkaline and acid phosphatase must be reduced by 50% on three separate occasions; and abnormal bone scan must improve. There must be a reduction in size or number of lesions as reported by the Department of Nuclear Medicine. For a patient to be considered a responder, an improvement in all abnormal parameters was required.

RESULTS

Eight of 39 patients (21%) achieved an objective response. The median duration of response was 28 weeks (mean, 30 ± 5.5). Five patients with bone metastasis responded; all had improvement in bone scan and acid phosphatase. Three patients with elevated alkaline phosphatase had a > 50% reduction in their increase. All four patients with pain had marked improvement in their symptoms.

A patient with retroperitoneal nodal metastasis causing massive lower-extremity edema had reduction in his acid phosphatase and improvement in his lymphatic obstructive symptoms.

Two patients with visceral metastasis responded. One had liver and omental metastasis documented by computerized tomography and the other had pulmonary metastasis.

Four of the eight responders had a performance sta-

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TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total	39
Median age in yrs (range): 67 (52-82)	
Performance status*	
0-1	17 (44)
2	10 (26)
3	6 (15)
4	6 (15)
Elevated acid phosphatase	32 (82)
Elevated alkaline phosphatase	27 (69)
Predominant site of metastasis	
Axial skeletal or nodal disease	17 (43)
Axial and extremity skeletal involvement	16 (41)
Pulmonary	3 (8)
Liver	3 (8)
Prior therapy	
Hormonal manipulation	39 (100)
Orchiectomy	29 (74)
Estrogens	39 (100)
Radiotherapy	24 (62)
Chemotherapy	25 (64)

*Eastern Cooperative Oncology Group criteria.

tus of 4. Three of the eight responding patients had been exposed to prior chemotherapy, and of those, two had obtained objective responses with doxorubicin, mitomycin, and 5-FU.

Toxicity

Toxicity data are summarized in table 2. No significant nausea and vomiting occurred. No motor neuropathy was noted. Cardiac complications occurred in four patients; all four patients were managed appropriately and treatment did not have to be discontinued. One patient developed angina on this third day of vinblastine, two had premature ventricular contractions (at Day 4 of therapy and 4 weeks after), and one developed second-degree heart block requiring insertion of a permanent pacemaker 3 weeks after therapy. The last patient had a history of triple coronary bypass, angina, and bifascicular block before vinblastine infusion began. The other three patients had symptomatic coronary artery disease before chemotherapy.

TABLE 2.—Toxicity

Toxicity	No. of courses
Total	133
Nadir leukocyte count ($\times 10^3$/ml)	
Median: 2.8	
Range: 0.2-6.6	
Nadir platelet count ($\times 10^3$/ml)	
Median: 184	
Range: 33-448	
Neutropenic infections	10
Cardiotoxicity	4
Arrhythmias	3
Angina	1
Ileus	1
Myalgias	1

DISCUSSION

Our experience indicates that vinblastine is an active agent in patients with prostate cancer. Responses were seen among patients with poor performance and heavy tumor burden. In addition, three responding patients had been exposed to prior chemotherapy.

Toxicity with continuous-infusion vinblastine was modest. Vinblastine was administered with portable infusion pumps, and no extravasation necrosis was seen. There were no deaths from toxicity.

Cardiac complications have been reported with vinca alkaloids (3-6). In four of our patients, cardiac symptoms were exacerbated after vinblastine infusion. The mechanism for this effect might be increased sensitivity of the myocardium to catecholamines, as has been documented in the rat model (7). Patients with symptomatic coronary artery disease should be observed closely as inpatients during vinblastine-infusion therapy. Angina or arrhythmias should be adequately controlled prior to the initiation of the vinblastine infusion.

In conclusion, we believe that vinblastine administered by continuous 5-day infusion is active in heavily pretreated prostate cancer patients and has acceptable toxicity. Patients with symptomatic coronary artery disease should be treated with caution, since they could have exacerbation of their symptoms during therapy.

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Cyclophosphamide/Etoposide: Effective Reinduction Therapy for Children With Acute Nonlymphocytic Leukemia in Relapse[1,2]

David K. Kalwinsky,* Gary V. Dahl, J. Mirro, Jr, and A. Thomas Look[3,4]

Reinduction therapy consisting of cyclophosphamide (250 mg/m² orally daily for 4 days) followed by etoposide (250 mg/m² iv daily for 3 days) was administered to 14 children with refractory or recurrent acute nonlymphocytic leukemia. Five complete remissions were achieved in eight patients who had relapsed in the bone marrow 1–27 months after cessation of initial therapy, which included anthracyclines, cytarabine, etoposide, and 5-azacitidine. Reinduction attempts were unsuccessful in patients who had failed to achieve an initial remission and in those whose relapses occurred while receiving therapy. Toxicity, including myelosuppression and mucositis, was within acceptable limits. This drug combination deserves further assessment in therapeutic protocols for patients with acute nonlymphocytic leukemia. [Cancer Treat Rep 69:887–889, 1985]

With the use of intensive multidrug primary therapy for acute nonlymphocytic leukemia (ANLL), second remissions are often difficult to obtain and of short duration (1). We report here the responses of 14 children with initially resistant or recurrent ANLL to the two-drug combination of etoposide (VP-16)/cyclophosphamide (Cyclo) (2). VP-16 has shown promising activity when combined with 5-azacitidine (5-Az) for the treatment of refractory or relapsed ANLL (3); however, the reported toxicity is severe and the therapeutic contributions of the individual drugs have been difficult to assess. Cyclo as a single agent has limited effectiveness against ANLL (1,4), but studies with L1210 murine leukemia suggest that it might potentiate VP-16 cytotoxicity (5). Our pharmacologic rationale was that Cyclo might recruit myeloblasts into S phase of the cell cycle (6), thereby enhancing cell kill by VP-16, which acts primarily on replicating DNA (7).

MATERIALS AND METHODS

Fourteen children with previously treated ANLL were enrolled from 1982 to 1984. Informed consent was given by each patient, and the protocol was approved by the institution's Clinical Trials Committee. All patients had been heavily treated before enrollment, 13 in a study designated AML-80 (8), which included constant-infusion cytarabine (ara-C), maximal doses of anthracyclines, and VP-16/5-Az, and one in an earlier clinical trial (9) that featured cytokinetic-based therapy. Six patients had also received constant-infusion high-dose interferon or high-dose methotrexate/amsacrine in unsuccessful attempts to induce second remissions. Type I induction failures (table 1) are defined as patients who continue to show circulating blasts despite aggressive induction chemotherapy with anthracyclines, ara-C, VP-16, and 5-Az (10).

Induction therapy consisted of oral Cyclo (250 mg/m²) for 4 days followed by VP-16 (250 mg/m²) given as a 45-minute iv infusion on Days 5–7. Bone marrow aspirates were obtained from all patients on Day 10 to evaluate antileukemic responses. In the absence of hypoplasia (< 5% blasts), a second 7-day course of therapy was administered. Patients who failed to achieve hypoplasia after two courses were removed from the study. For those patients achieving complete remission, continuation therapy consisted of alternating monthly courses of Cyclo/VP-16 and 6-thioguanine (200 mg/m² orally daily × 5)/ara-C (200 mg/m² sc by constant infusion × 5 days) for 1 year.

Serial bone marrow aspirates were obtained before

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TABLE 1.—Clinical characteristics and response to therapy

	No. of patients
Total	14
Male	10
Female	4
Median age in yrs (range): 14.5 (0.7-20)	
FAB type*	
M-1/M-2	10
M-3	0
M-4/M-5	4
Treatment response	
Relapse off treatment (n = 8)	
Hypoplasia	5
Complete remission	5
Relapse on treatment (n = 3)	
Hypoplasia	2
Complete remission	0
Type I induction failure (n = 3)	
Hypoplasia	0
Complete remission	0

* All patients were classified by morphology, with cytochemical confirmation of myelocytic or monocytic differentiation. French-American-British (FAB) subtypes were concordant at diagnosis and relapse.

treatment, after the fourth dose of Cyclo, and 24 hours after the first dose of VP-16, for flow cytometric analysis of cell cycle changes induced by these two drugs (11).

RESULTS

Five of the eight patients relapsing after cessation of therapy achieved a second complete remission (table 1). In the remaining groups (type I induction failures and relapses on therapy), there were no additional complete remissions, despite evidence of marrow hypoplasia in two patients. Characteristics of the five complete responders are summarized in table 2. One child had monocytic ANLL (FAB type M-5), while the remainder had M-1 or M-2 disease. Two of these patients remain free of leukemia for 14+ to 16+ months after attaining a second remission and are off treatment for a second time.

In most patients it was possible to administer chemotherapy on an outpatient basis. Patients were hospitalized only when necessary for management of complications of bone marrow failure. With the exception of one child who had fungal pneumonitis and anthracycline-induced congestive heart failure, clinical status at the time of enrollment was satisfactory. Pancytopenia, the major form of toxicity, was manifested as neutropenia (< 500 cells/mm^3) persisting for 23-37 days (median, 30) and thrombocytopenia (< 30,000 cells/mm^3) persisting for 0-44 days (median, 21). Mild mucositis was noted in four patients after they completed their second induction course, but total parenteral nutrition was not required. Six patients were hospitalized during marrow aplasia for *Candida* pneumonia, systemic herpes, or fever with neutropenia. No metabolic, hepatic, renal, or cardiac abnormalities were noted.

After administration of Cyclo, four patients showed a ⩾ 50% reduction in numbers of circulating myeloblasts, but for all others three doses of VP-16 were needed to clear blasts from the peripheral blood. There was little indication that recruitment of malignant cells into S phase by Cyclo had influenced whether or not a patient achieved complete remission. Only one complete responder showed an increased percentage of S-phase blasts after receiving the drug, compared with two of five nonresponders. By contrast, complete responders showed 1.3-fold to 1.8-fold and 1.5-fold to 5.5-fold decreases in percentages of S- and G_2 + M-phase cells, respectively, after VP-16 therapy, possibly reflecting the extent of cell kill by this drug. Such decreases were not observed among nonresponders.

DISCUSSION

The combination of Cyclo/VP-16 appears to be effective, well-tolerated reinduction therapy for ANLL recurring after cessation of therapy. It has the advantage of mild mucosal toxicity without the complications usually associated with anthracycline compounds/ara-C (12) or VP-16/5-Az (3), and it can be administered to patients whose cardiac tolerance precludes continued use of anthracyclines.

The failure of Cyclo to eradicate circulating myeloblasts in most patients or to recruit an appreciable per-

TABLE 2.—Characteristics of complete responders

Patient No.	FAB type	Time off initial treatment (mos)	Previous treatment*	Duration of 2nd remission (mos)
1	M-1	6	A, B, C, D	3
2	M-1	4	A	9
3	M-2	27	E, F	16+
4	M-2	11	A, B, C	14+
5	M-5	7	A, B, C	9

* A = sequential intensive chemotherapy (ref 8); B = high-dose methotrexate and amsacrine; C = continuous-infusion interferon; D = allogeneic bone marrow transplantation; E = cytokinetically based chemotherapy (ref 9); and F = 6-mercaptopurine, vincristine, methotrexate, and prednisone.

centage of blasts into S phase indicates that VP-16 was the more active drug in this combination. The general correlation between changes in percentage of S-phase cells and response to therapy following VP-16 administration supports the efficacy of the epipodophyllotoxin. Nonetheless, Cyclo appeared to have made a contribution to the observed antileukemic responses, which clearly exceeded those one could expect from the use of VP-16 alone (13,14).

Our results indicate that patients who do not achieve marrow hypoplasia during first-line therapy, including VP-16/5-Az, are unlikely to respond to an additional VP-16-containing combination. Patients who develop disease recurrence while receiving initial intensive treatment likewise do not respond well to Cyclo/VP-16. The greater responsiveness of patients who relapsed after cessation of therapy is consistent with experience in relapsed acute lymphoblastic leukemia (15).

Most of our complete responders had myeloblastic disease, underscoring the effectiveness of this combination for the spectrum of ANLL, not merely monocytic cases. The durations of second complete remission in two patients (14+ and 16+ months) are unusual in ANLL and most encouraging. We suggest that Cyclo/VP-16 might be an effective addition to first-line induction regimens for patients with ANLL.

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Effectiveness of Cisplatin in the Treatment of Anal Squamous Cell Carcinoma[1]

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The therapeutic activity of cisplatin was explored in three consecutive patients with locoregional squamous cell carcinoma of the anus who received no prior therapy. One complete response and two partial responses were achieved. These data, although preliminary, strongly suggest that this disease is sensitive to cisplatin, and further trials are indicated to assess the precise role of this drug in the overall management of anal carcinoma. [Cancer Treat Rep 69:891–893, 1985]

In the last decade, squamous cell carcinoma of the anus has been shown to be responsive to the combined modality approach including chemotherapy and radiation therapy (1). More than half of the patients who were treated preoperatively in this manner achieved a pathologically proven complete remission (2). In these studies, chemotherapy included 5-FU and mitomycin and was delivered concomitantly with irradiation. Accordingly, the role of chemotherapy alone was not adequately assessed. In this report, we attempted to explore the role of cisplatin (DDP) in the treatment of squamous cell carcinoma of the anus independently from radiation therapy. DDP was selected because of a previous report demonstrating its activity against this disease (3) and because of its established activity against a variety of squamous cell neoplasms. Three consecutive patients were treated.

CASE REPORTS

Patient 1

Patient 1 was a 66-year-old woman who first presented to the American University of Beirut Medical Center (AUBMC) in September 1983 with a 6-month history of rectal bleeding. Rectal examination then revealed a mass (4 × 3 cm) just below the dentate line and an adjacent pedunculated tumor (2 × 1 cm). There was no fixation to underlying tissues. Inguinal lymph nodes were not palpable. A computerized axial tomographic (CAT) scan of abdomen and pelvis revealed no evidence of disease outside the primary locale of the tumor and in particular there was no evidence of pelvic adenopathy. Chest x-ray was normal. Biopsy of this lesion revealed well-differentiated squamous cell carcinoma. Also on physical examination, there was a suspicious mass (3 × 2 cm) in the upper outer quadrant of the right breast. A biopsy of this mass confirmed the diagnosis of primary breast adenocarcinoma and the patient underwent right radical mastectomy on October 8, 1983. Pathology revealed a primary tumor (2 × 1.8 cm) and 16 negative axillary lymph nodes. Postoperatively, the patient was referred for treatment of the anal lesion with chemotherapy and radiotherapy. We decided not to give both modalities concomitantly and to evaluate the role of DDP alone in the treatment of this disease. Accordingly, the patient was admitted to the hospital on October 26 to receive the first cycle of treatment. DDP was given at a dose of 20 mg/m^2/day as a continuous 24-hour infusion for 5 consecutive days; courses were repeated every 4 weeks. On Day 1 of the second cycle, rectal examination revealed a 50% decrease in the size of the tumor. On Day 1 of the third cycle, rectal examination revealed no palpable evidence of residual disease. Three weeks after the cessation of the third cycle, rectal examination was again negative and the site of the initial tumor was biopsied. Pathology revealed no evidence of residual disease and the patient was referred for radiation therapy. This therapy was given to the anal region in 29 sessions, to a cumulative tumor dose of 6000 rads, and was completed on May 22, 1984. No further

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therapy was given after the completion of radiation therapy. The patient remained free of disease as of August 1984.

Patient 2

Patient 2 was a 60-year-old man who first presented to AUBMC in September 1983 with a 6-month history of rectal bleeding, pain on defecation, and constipation. Rectal examination then revealed a fungating mass (3 × 1 cm) at the anorectal junction. Inguinal lymph nodes were not palpable. Biopsy of the anal mass revealed well-differentiated squamous cell carcinoma. Chest x-ray and iv pyelogram were normal. CAT scan of abdomen and pelvis revealed a fungating mass filling the anal region and extending up into the rectum above the levator ani. The wall of the rectum was thickened with concentric narrowing of its lumen. There was no distinct interphase between the lowermost portion of the prostate and the anal mass. Also, there was minimal thickening of the levator ani muscles. There was no evidence of pelvic adenopathy. In addition, the CAT scan revealed a mass (3 cm) in the left adrenal gland (metastasis? adenoma?). The patient was referred for treatment with chemotherapy alone to be followed by radiation therapy. Chemotherapy was initiated on September 8, 1983, and the first treatment course consisted of DDP, methotrexate, vincristine, and bleomycin. DDP was given at a dose of 20 mg/m^2/day as a continuous 24-hour infusion for 5 consecutive days; methotrexate was given at a dose of 50 mg/m^2 iv on Days 1 and 15; vincristine was given at a dose of 1.4 mg/m^2 iv on Day 1; and bleomycin was given at a total dose of 15 mg im on Days 1, 5, and 15. As a result of this treatment, the patient developed neutropenia-associated sepsis, which was treated adequately with antibiotics. Accordingly, all drugs other than DDP were discontinued and four more cycles of DDP were given. The second cycle of DDP was initiated on Day 35 of the first cycle, and rectal examination then revealed evidence of an approximate 25% decrease in the size of the original tumor. DDP was now given at a dose of 50 mg/m^2/day as a 30-hour infusion for 2 consecutive days. This modification in the dose schedule of DDP was deemed necessary to allow the patient to receive treatment in the outpatient department, since he refused further hospitalization. DDP cycles were repeated every 4 weeks. On Day 1 of the fourth cycle, rectal examination and CAT scan of pelvis both revealed a > 90% decrease in the size of the tumor. The patient was lost to follow-up after receiving half the dose of the fifth cycle.

Patient 3

Patient 3 was a 63-year-old man who first presented to AUBMC in October 1983 with a history of rectal bleeding of 18 months' duration and a weight loss of 30 kg. Rectal examination then revealed an anal mass (15 × 10 cm) protruding through the anal orifice. In addition, the patient had bilateral enlarged and hard inguinal lymph nodes. Biopsy of the anal mass revealed well-differentiated squamous cell carcinoma. Diversion colostomy was performed on October 15, 1983, and there was no gross evidence of disease beyond the primary tumor. Because of the presence of bilateral inguinal lymphadenopathy, the patient was treated with chemotherapy alone. DDP was given at a dose of 50 mg/m^2/day as a 3-hour infusion daily for 2 consecutive days; courses were repeated every 4 weeks. The first cycle was initiated on November 3, 1983. The first re-evaluation was done on December 7, 1983, and it revealed a > 50% decrease in the size of the tumor. On completion of the third and last cycle of DDP, rectal examination revealed a > 75% regression in disease. The patient was then lost to follow-up.

DISCUSSION

Our data suggest that DDP is an effective agent in the treatment of squamous cell carcinoma of the anus. Of the three consecutive patients whom we treated, one achieved complete response and two achieved good partial response. The latter two patients were lost to follow-up while their disease was still responding to DDP. Although the first cycle of treatment for Patient 2 included drugs other than DDP, we tried to evaluate the therapeutic impact of DDP alone by giving four more cycles of the drug without the other agents. These four cycles produced an additional 50% tumor regression. Baseline evaluable parameters for DDP alone were determined 20 days after the completion of the first cycle. Obviously, our brief report does not provide data as to the impact of DDP therapy on duration of response, pattern of relapse, and survival.

Until recently, the conventional treatment of locoregional squamous cell cancer of the anal canal has been primarily surgical, with most patients undergoing abdominoperineal resection. Because this surgery was associated with a low 5-year survival (< 50%) (4), a high locoregional relapse rate (5), late complications, and the discomfort of permanent colostomy, new modalities of treatment were recently introduced. In 1974, Nigro et al (1) first reported on the concomitant use of radiation therapy and chemotherapy with 5-FU plus mitomycin, with encouraging results. Since then, other investigators reported more impressive response rates (> 75% of patients achieving complete response) (6). These results made the role of surgery controversial. However, in all of these studies, radiation therapy and chemotherapy were given either concomitantly (1,6) or chemotherapy was given just before radiation

therapy was initiated (2). Accordingly, the role of chemotherapy alone could not be adequately assessed in any of these studies. In addition, the concomitant use of chemotherapy and radiation therapy was associated with a high rate of complications including myelosuppression, severe gastrointestinal toxicity, perianal reaction, and proctitis (6). Therefore, we would like to advocate the use of chemotherapy alone prior to irradiation. In our opinion this is important for two reasons: (*a*) it is crucial to adequately assess the effectiveness of chemotherapy alone without irradiation in the treatment of this disease, and (*b*) patients who achieve complete response or good partial response with chemotherapy alone may not need conventional high doses of irradiation, which are frequently associated with local complications.

In summary, our data, although preliminary, are highly suggestive that squamous cell cancer of the anus is very sensitive to DDP. However, a larger number of patients should be initially treated with DDP alone in an adequate dose to further assess the precise role of this agent in the overall management of this disease.

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Recombinant Leukocyte Alpha Interferon in Advanced Ovarian Carcinoma[1,2]

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Recombinant leukocyte alpha interferon (rIFN-αA; Hoffmann-La Roche, Inc) was administered to 15 patients with recurrent or persistent ovarian carcinoma. All patients had been previously treated with surgery and combination chemotherapy including cyclophosphamide (15 patients), doxorubicin (14), and cisplatin (14). Three patients had also previously undergone radiation therapy. At the start of therapy the largest tumor size was ≤ 2 cm in four patients and > 2 cm in 11. Interferon was administered im three times weekly for 8 weeks at a dose of 20 × 10^6 units/m^2, with average drug levels of 2267 pg/ml 6 hours after im injection. In three patients (20%), the dose had to be reduced by 50% because of drug toxicity. Side effects included fever (> 101°F) in 12 patients, fatigue in ten, headache in two, diarrhea in two, and reversible myelosuppression in five. Of the 15 patients, one had mixed response lasting 12 weeks, two had stable disease of 8 weeks' duration, and 12 had disease progression. [Cancer Treat Rep 69:895–896, 1985]

Although ovarian carcinoma responds to cytotoxic chemotherapy and radiation therapy, a 5-year survival free from recurrent disease remains uncommon. Additional therapeutic modalities might provide more effective management. Interferon has been shown to be active in a number of human tumors, and previous reports suggest that it may be active against ovarian carcinoma as well (1–3). This study was undertaken to assess the efficacy and toxicity of recombinant leukocyte alpha interferon in patients with recurrent or persistent ovarian carcinoma.

METHODS

Fifteen patients with recurrent or persistent epithelial carcinoma of the ovary were entered in this protocol. The clinical data for each patient are presented in table 1. The median age was 57 years. All tumors were originally stage III or IV. Histologic classification of the tumors included seven papillary serous, one endometrioid, one clear cell, and six undifferentiated adenocarcinomas. One patient had a grade 1 tumor, four had grade 2 tumors, and ten had grade 3 tumors. All patients had previously undergone laparotomy at least once and had been treated with combination chemotherapy including cyclophosphamide (15 patients), doxorubicin (14), and cisplatin (14). In addition, three patients had received radiation therapy. At the start of therapy the largest measurable lesion was ≤ 2 cm in four patients and > 2 cm in 11. After informed consent was obtained from each patient, recombinant leukocyte alpha interferon (rIFN-αA; Hoffmann-La Roche, Inc), 20 × 10^6 units/m^2, was administered im three times weekly for 8 weeks. Standard definitions were used in the characterization of disease response.

RESULTS

Of the 15 patients, three were withdrawn from the protocol after 1 week due to rapidly progressive disease manifested by small bowel obstruction. Among the 12 patients who completed 4 weeks of treatment, one had mixed response lasting 12 weeks, two had stable disease of 8 weeks' duration, and nine had disease progression. In the patient with mixed response, the tumor was at the vaginal apex and was found on clinical examination to have decreased in size by > 50%. However, a 4-cm lesion was also evident in the liver, and this lesion remained stable in size on serial computer tomographic examinations.

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TABLE 1.—Patient characteristics and clinical response to im interferon

Patient No.	Age (yrs)	Histology (adenocarcinoma)	Grade	Previous radiotherapy	Site of measured lesion	Size (≤ 2 cm)	Duration of therapy (wks)	50% reduction in dose	Response*
1	69	Undifferentiated	3	Yes	Vagina/ liver		12	Yes	MR
2	57	Undifferentiated	3		Abdomen		8		NR
3	52	Papillary serous	3	Yes	Pelvis		8		SD
4	58	Endometrioid	2	Yes	Pelvis		8	Yes	SD
5	53	Papillary serous	3		Lung		5		NR
6	64	Papillary serous	3		Pelvis	Yes	8	Yes	NR
7	31	Clear cell	2		Pelvis		8		NR
8	43	Papillary serous	1		Abdomen	Yes	8		NR
9	57	Undifferentiated	2		Abdomen	Yes	1		NR
10	63	Papillary serous	2		Abdomen	Yes	4		NR
11	63	Undifferentiated	3		Abdomen		1		NR
12	61	Undifferentiated	3		Abdomen		1		NR
13	32	Undifferentiated	3		Lung		4		NR
14	53	Papillary serous	3		Liver		8		NR
15	57	Papillary serous	3		Abdomen		4		NR

*MR = mixed response; NR = no response; and SD = stable disease.

Interferon levels averaged 2267 pg/ml 6 hours after im injection.

Toxic effects are shown in table 2. The most common manifestations of toxicity were fever and fatigue occurring in ten and 12 patients, respectively. There were no renal, hepatic, or neurologic toxic effects. Neutropenia was observed in five patients and thrombocytopenia was observed in three, with one severe case in each category. In three patients, the dose of interferon had to be reduced by 50% due to toxicity. No patient died while receiving this agent.

DISCUSSION

Earlier studies have suggested that interferon has activity against ovarian cancer (1-3). In this study, interferon was given to 15 patients previously treated for epithelial ovarian cancer. There were no complete responses and only one mixed response. Since interferon was not administered as primary therapy after surgical cytoreduction but was given after each patient had already exhibited tumor progression despite chemotherapy, most patients had large tumors. However, none of the four patients with tumors ≤ 2 cm responded to interferon therapy.

In studies utilizing nonrecombinant interferons, response rates have varied from 7% to 20%. Although the apparent lack of activity observed in our study may relate to the small number of patients and their previous treatment, it is possible that polygenic nonrecombinant interferon may be superior to the recombinant preparations for this particular tumor. A similar trend has been observed with the use of interferon in the treatment of patients with breast cancer (4).

TABLE 2.—Toxicity with im interferon

Toxicity	No. of patients (n = 15)
Fever*	10
Fatigue	12
Headache	2
Diarrhea	2
Neutropenia†	
Grade 1	1
Grade 2	3
Grade 3	1
Thrombocytopenia‡	
Grade 1	1
Grade 2	1
Grade 3	1

*> 101°F.

†Granulocyte count/mm^3: grade 1 = 1000-1499; grade 2 = 750-999; and grade 3 = 500-749.

‡Platelet count/mm^3: grade 1 = 75,000-100,000; grade 2 = 50,000-74,999; and grade 3 = 25,000-49,999.

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Phase I Study of High-Dose Cytarabine and Cisplatin in Patients With Advanced Malignancy[1,2]

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Nineteen patients with advanced malignancy participated in a phase I trial of high-dose cytarabine (ara-C) and cisplatin in combination. Dose and schedule were based on laboratory data indicating synergy for concurrent use of these drugs. Cisplatin (100 mg/m^2) was administered during the 2nd and 3rd hours of a 3-hour ara-C infusion. The ara-C dose was escalated in subsequent patients following a starting dose of 1 g/m^2. Two brief responses were noted. The study was terminated prematurely due to protracted (several weeks) nausea, occasional vomiting, and severe lassitude. [Cancer Treat Rep 69:897–899, 1985]

In vivo studies using the LoVo cell line have demonstrated pharmacologic synergy between cisplatin and cytarabine (ara-C). However, for synergy to be apparent, very high concentrations of ara-C (10^{-3} to 10^{-2} M) were required, while the concentrations of cisplatin (2–5 μg/ml) were those achievable with standard clinical doses (1). Similarly, greater than additive cytotoxicity against a human lymphoma cell line was seen with the concurrent use of cisplatin and ara-C (10^{-4} to 10^{-3} M) (2). Because of these laboratory observations, a phase I study of cisplatin with escalating doses of ara-C was undertaken.

MATERIALS AND METHODS

A total of 19 patients with a variety of malignancies received 31 courses of the combination. All patients had failed standard or higher priority treatment programs, and all gave informed consent in accord with federal, state, and institutional guidelines.

Initial prestudy evaluation included history and physical examination; hemogram; blood chemistry profile including liver function tests, serum creatinine, and creatinine clearance; and ECG. Patients with measurable disease had appropriate sites followed for response. Cbc and platelet counts were done weekly following treatment.

All patients received a 3-hour infusion of ara-C in escalating doses (1, 2, 3, and 4 g/m^2). At least three patients were studied at each dose level. Cisplatin (100 mg/m^2) was given concurrently with ara-C during the 2nd and 3rd hours. Treatment was started after overnight iv hydration with 500 ml of 5% dextrose in one-half normal saline plus 20 mEq of KCl given at 150 ml/hour. Thirty minutes before cisplatin administration, 500 ml of 5% dextrose in one-half normal saline was given, followed by rapid infusion of 12.5 g of mannitol in 50 ml of 5% dextrose. Cisplatin was then infused over 30–60 minutes and immediately followed by 1000 ml of 5% dextrose in normal saline over 1½ hours. Hydration was then maintained by giving 5% dextrose in one-half normal saline with 20 mEq of KCl and 8 mEq of $MgSO_4$ (in 500 ml) at 125 ml/hour for at least 8 hours or until nausea and vomiting resolved.

RESULTS

Patients entered in the study had a variety of malignancies, including colorectal cancer (four patients); lymphoma (four); esophagus and gastric cancers (three); lung cancer (two); melanoma (two); sarcoma (two); and renal and breast cancers (one each). The mean age was 50 years (range, 28–66), with 11 males and eight females. The mean number of prior chemotherapy regimens was four (range, zero to eight). The performance status (Zubrod) was 0–1 in 13 patients and 2–3 in six. Patients placed on

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protocol had an average of 1.5 courses (range, one to three) of prior treatment.

Toxicity is shown in table 1. Leukopenia < 1000 cells/μl was not noted. Two patients, however, had platelet counts < 10,000/μl. Both of these patients had been heavily treated; therapy included a nitrosourea (lomustine). One patient required platelet support for several weeks, while the other had a gradual return of platelet counts to subnormal levels over a 3-week period. None of the hematologic effects resulted in clinical complications.

Nausea and vomiting were moderate during treatment; however, increased symptoms were noted at ara-C doses of 4 g/m^2. After treatment, protracted nausea and occasional vomiting occurred in all patients, lasting from 7 to 10 days and occasionally longer. One patient required rehospitalization for emesis control.

Severe nephrotoxicity was noted in two patients. Neither patient had predisposing factors, and in both, toxicity occurred after the second course. One patient with rectal carcinoid, liver metastases, and carcinoid syndrome had a rise in creatinine from 1.3 to 5.5 mg/dl. His creatinine is now 2.5 mg/dl several weeks after therapy. A second patient had an elevation of creatinine from 1.0 to 12.8 mg/dl. He developed symptoms and signs of uremia and was intensively hemodialyzed for 2 weeks; his creatinine slowly decreased to 3.2 mg/dl 2 months after treatment.

A woman with extensive breast cancer, who had had intensive prior chemotherapy, died 4 days after treatment. Twenty-four hours following therapy, she was in mild heart failure. Chest x-rays showed increased interstitial markings and ECG was unchanged. One day later, marked elevation of SGOT and LDH was noted, and the patient developed a low-grade fever. She was presumed to have sepsis, appropriate cultures were obtained, and antibiotics were started. The patient continued to deteriorate and died 4 days after receiving 4 g/m^2 of ara-C as her starting dose. Consent for postmortem examination was refused.

The major dose-limiting toxic effects were lassitude and fatigue. All patients had severe malaise for 1–3 weeks after therapy and many refused to accept further treatment. This protracted lassitude appears to be unique to this high-dose ara-C/cisplatin combination and has not been described in other patients treated with platinum and lower doses of ara-C. In addition, fever without evidence of infection, as high as 103°F, was noted in several patients. At least five patients complained of hearing loss. Pulmonary toxicity, conjunctivitis, and neurotoxicity were not observed. Diarrhea was seen on two occasions in the same patient following therapy but was thought to be secondary to metoclopramide.

Of the 19 patients, one achieved partial response. This patient with diffuse histiocytic lymphoma had a > 50% shrinkage of focal defects on liver scan following his first course of therapy. He received a total of three courses but was then noted to have progressive disease. A second patient with gastric carcinoma and skin involvement had stability of two lesions but complete resolution of a third after two courses of therapy. Because of severe toxicity, however, the patient refused further treatment.

DISCUSSION

The doses and schedule used in this study were poorly tolerated and noncompliance became a significant factor in continuing therapy. Hearing loss appeared to be more

TABLE 1.—Maximum toxicity/course

	No. of courses			
	1 g/m^2	2 g/m^2	3 g/m^2	4 g/m^2
Total	8	4	7	12
Wbc count (cells/μl)				
≥ 4000	5	3	3	6
3000–4000	—	—	1	2
2000–3000	2	1	1	1
1000–2000	—	—	2	1
< 1000	—	—	—	—
Not done	1	—	—	2
Platelet count (cells/μl)				
> 100,000	4	2	2	6
50,000–100,000	3	2	2	1
25,000–50,000	—	—	1	3
< 25,000	—	—	2	—
Not done	1	—	—	2
Nausea/vomiting				
None	—	2	4	—
Mild	5	2	1	7
Moderate	3	—	2	2
Severe	—	—	—	3

frequent than that usually observed with cisplatin alone or in other combinations, and it is possible that the ara-C potentiated the ototoxicity of cisplatin. Protracted nausea and vomiting and severe malaise proved to be dose-limiting at all levels and represents an unusual and previously unreported side effect of either drug when given singly. Hematologic toxicity was acceptable and not dose-limiting. The study was closed after it became obvious that prolonged treatment with this regimen was poorly tolerated.

Lopez et al (3), in a similar phase I trial involving six patients, did not report protracted toxicity when cisplatin immediately preceded high-dose ara-C. In their trial, ara-C (3 g/m^2) was given every 12 hours for two doses via a 1-hour infusion. Ara-C was preceded by cisplatin at a dose of either 75 mg/m^2 before the first dose or 40 mg/m^2 before each dose of ara-C. Possible differences accounting for this reported lack of protracted toxicity include sequence of drug administration, short infusion time of ara-C, and the slightly lower doses of cisplatin.

Further phase I studies using different doses and schedules of ara-C and cisplatin will be necessary to define phase II regimens suitable for clinical trial that maintain pharmacologic parameters for synergy without protracted toxicity. When possible, such schedules should be considered for testing in suitable animal models to determine any unusual or unexpected toxicity.

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Phase II Trial of Elliptinium in Advanced Renal Cell Carcinoma[1]

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Forty patients with advanced renal cell carcinoma were treated with elliptinium by a weekly infusion of 100 mg/m². Of 38 evaluable patients, five had an objective response (13.2%). Average response duration was 8 months (range, 5–11). The major dose-limiting toxic effect was induction of antielliptinium antibodies, with the risk of intravascular hemolysis. Elliptinium has modest activity in advanced renal cell cancer and does not produce myelosuppression. [Cancer Treat Rep 69:901–902, 1985]

Elliptinium, a DNA-intercalating agent, is a synthetic quaternary derivative of the indole alkaloid ellipticine (1). Its antineoplastic activity has been demonstrated in experimental preclinical studies (2) and confirmed in human breast cancer (3). An earlier report of 12 patients with advanced renal cell cancer showed objective responses in three patients (4). The present phase II study represents an extension of these preliminary observations, to now include 40 patients.

PATIENTS AND METHODS

Adult patients with histologically confirmed advanced renal cell carcinoma were entered in this study. Only patients with two-dimensionally measurable disease were included. Irradiated metastases, pleural effusions, ascites, and bone metastases were not accepted for measurement of objective response. Additional eligibility criteria included wbc count > 4000/mm³, platelet count > 100,000/mm³, normal serum creatinine, normal hepatic function, and no chemotherapy or hormonal therapy for 3 weeks prior to initiation of elliptinium treatment.

Elliptinium was administered weekly at a dose of 100 mg/m² in 500 ml of 5% dextrose in water as a 1-hour iv infusion. Prior to each infusion, blood samples were obtained for routine biochemical and hematologic testing and, later in the study, for measurement of antielliptinium antibodies (5).

The World Health Organization (WHO) response criteria (6) were employed. Response duration was measured from the date of the start of therapy. Patients were considered evaluable after having received four weekly courses of elliptinium.

RESULTS

Forty patients were entered in the study between February 1979 and December 1983 at the Institut Gustave-Roussy (Villejuif, France). Thirty-eight patients were evaluable for response and toxicity. Two patients were considered nonevaluable: one because of death due to progressive disease after only 3 weeks of treatment and the other because of failure to document metastatic disease sites. Pretreatment patient characteristics are shown in table 1.

Objective responses were observed in five of the 38 evaluable patients (13.2%). A complete response of 8 months' duration was achieved in a patient with multiple bilateral pulmonary parenchymal metastases. A second patient with multiple biopsy-proven subcutaneous metastases, varying in size from 0.5 to 2 × 6 cm², had an initially inoperable primary tumor. Elliptinium treatment resulted in the complete disappearance of soft tissue disease. The patient was then able to undergo a radical nephrectomy, with removal of an almost completely necrotic renal cell carcinoma. This surgical complete response lasted 22 months. Partial responses were achieved in two patients with multiple pulmonary parenchymal metastases (duration, 5 and 12 months, respectively) and in one patient with subclavicular lymph node metastases (duration, 6 months). The me-

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TABLE 1.—Pretreatment patient characteristics

Characteristic	No. of patients
Total/evaluable	40/38
Median age in yrs (range): 55 (19-75)	
Sex: male/female	28/12
Performance status*	
0-1	23
2-3	17
Initial nephrectomy	36
Sites of metastatic disease	
Lung	29
Bone	7
Lymph nodes	3
Pleura	3
Liver	3
Soft tissue	2
Brain	1
Prior therapy for metastases	
None	22
Chemotherapy	10
Hormonal therapy	6
Radiation therapy	2
Surgery	1

*Eastern Cooperative Oncology Group criteria.

dian duration of the objective responses was 8 months (range, 5-22). Eighteen patients had either minor responses or stable disease, while 15 had clear disease progression.

There was a notable lack of myelosuppression during the study. Immunemediated hemolysis occurred in three patients early in the trial, associated with positive antielliptinium antibodies. Subsequently, regular monitoring of antielliptinium antibody titers revealed positive titers in five of 18 patients (28%). No further hemolytic episodes occurred. Nonhematologic toxic effects encountered were generally mild, with the exception of local pain and phlebitis in seven of 18 patients (39%) in whom peripheral venous infusion sites were employed. Xerostomia, generally mild, occurred in 28 of the 38 patients (74%). Other toxic effects included nausea and vomiting (26%), fever and chills (16%), and muscle cramps (13%). No significant disturbances in renal function were noted.

DISCUSSION

Renal cell cancer remains one of the most unresponsive tumors to chemotherapy. Response rates to a wide variety of agents, both alone and in combination, tend to be in the disappointing range of 0%-15%, with vinblastine being the most active single agent (7).

The present report of elliptinium activity in advanced renal cell cancer represents extension of a preliminary study in which antitumor responses (complete and partial) were seen in three of 12 patients (25%) (4). Among 38 evaluable patients now included, there are five responses (two complete; three partial), for an overall response rate of 13.2%. Four of the responses occurred in the subgroup of 25 patients who had had no prior hormonal therapy or chemotherapy. Four of the responders were in the subgroup of 23 patients, with an ECOG performance status of 0 or 1. Among the 16 patients in the subgroup with both of these favorable pretreatment characteristics, four responses were achieved (25%).

Weekly elliptinium administration was reasonably well-tolerated in the present study. The major dose-limiting toxic effect of this schedule appeared to be induction of antielliptinium antibodies and the resultant risk of hemolytic anemia. However, when screening for antibodies was performed regularly prior to each treatment, with treatment being discontinued in the case of a positive test (8), no hemolysis was observed.

In conclusion, elliptinium must be considered as an agent having a modest level of activity in advanced renal cell cancer, particularly in patients with good performance status who have not had prior chemotherapy or hormonal therapy. Its lack of myelotoxicity makes elliptinium an appealing candidate to include in combination chemotherapy with other active agents. It is anticipated that schedule manipulations might lessen or eliminate some of the observed toxic effects.

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Bleomycin, Mitomycin, and Cisplatin Therapy for Advanced Squamous Carcinoma of the Uterine Cervix: A Phase II Study of the Northern California Oncology Group[1,2]

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Twenty-eight patients with advanced squamous carcinoma of the uterine cervix received cisplatin, bleomycin, and mitomycin after failure of surgery and/or irradiation to control disease. Six patients (21%) achieved responses (two complete; four partial), ranging from 3 to 7+ months. Toxicity was acceptable for most patients; however, dose reduction because of myelosuppression was frequently required. Bleomycin was delivered by continuous iv infusion, and no significant pulmonary toxicity was observed. Although this combination of drugs has activity in advanced squamous carcinoma of the uterine cervix, the addition of cisplatin to bleomycin and mitomycin did not significantly increase the clinical response rate. [Cancer Treat Rep 69:903–905, 1985]

Although some investigators have reported encouraging response rates to combination chemotherapy in recurrent or metastatic squamous carcinoma of the cervix, little improvement has been seen in response duration or survival prolongation (1). A previous Northern California Oncology Group study (NCOG 5C81) reported a remission rate of 22% for the combination of bleomycin and mitomycin in patients with advanced cervical cancer (2). This NCOG phase II study (5C91) assesses the effect of the addition of cisplatin to bleomycin and mitomycin. Cisplatin was added because of its activity as a single agent in this disease (3), as well as its nonoverlapping toxicity with the other two drugs.

In addition, bleomycin was administered by continuous infusion rather than by iv bolus. Both an increase in antitumor efficacy and a decrease in pulmonary toxicity have been reported with the administration of bleomycin by infusion (4,5).

MATERIALS AND METHODS

Patients with locally recurrent metastatic squamous carcinoma of the cervix were eligible for study once their disease was no longer amenable to resection or radiation therapy. Other entrance criteria were (*a*) Karnofsky performance status $\geq$ 60%; (*b*) measurable disease by physical examination, x-ray, computerized tomographic scan, or sonogram; (*c*) serum creatinine $\leq$ 1.5 mg/dl or creatinine clearance $\geq$ 50 ml/minute; and (*d*) normal peripheral blood leukocyte and platelet counts. Patients who had received prior chemotherapy were excluded, as were those with acute bleeding or infectious complications. Informed consent was obtained from all patients.

Chemotherapy consisted of bleomycin (15 units/m^2/day on Days 1–3 as a continuous iv infusion, repeated every 21 days to a maximum dose of 270 units/m^2); cisplatin (50 mg/m^2/day over 2 hours on Day 4 with pre- and post-hydration, repeated every 21 days); and mitomycin (10 mg/m^2 as an iv bolus on Day 1, repeated every 42 days). Some patients received bleomycin infusions as outpatients using a disposable, portable infusion pump with a 24-hour capacity (Travenol Infusor, Travenol Laboratories, Deerfield, IL). Doses were reduced or withheld for leukocyte counts $<$ 3000/mm^3, platelet counts $<$ 100,000/mm^3, or creatinine clearances $<$ 50 ml/minute.

Tumor response was scored as either a partial response

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(a > 50% reduction in the product of the perpendicular tumor measurements) or as a complete response (the complete disappearance of all measurable and evaluable sites of disease).

RESULTS

All 28 patients were evaluable for toxicity and response. Two other patients were declared ineligible because of inadequate renal function and absence of measurable disease. Patient characteristics are shown in table 1. The overall response rate was 21% (six responses among 28 patients; two complete and four partial), and durations of response ranged from 3 to 7+ months (median, 4). Three patients with mixed responses are not included in this overall response rate. Responses occurred in only one of 11 patients (9%) with tumor confined to the pelvis. Four of 14 patients (29%) with both pelvic and extrapelvic tumor responded in all sites. An additional three of these patients responded in extrapelvic sites only, while pelvic tumor remained stable. One of three patients with extrapelvic disease only responded to treatment. Thus, responses in extrapelvic disease sites were seen in eight of 17 patients (47%), but pelvic disease regressed in only five of 25 patients (20%).

The median number of chemotherapy cycles (a cycle defined as 6 weeks) received by patients in this study was two (range, 0.5–3.5). Dose reductions of cisplatin and mitomycin because of myelosuppression occurred in almost all patients who received more than one cycle of therapy. However, only one patient experienced granulocytopenia of < 1000 cells/mm^3; no significant thrombocytopenia was encountered. Moderate to severe nausea was frequent in association with chemotherapy, and three patients developed alopecia. Other significant toxic effects (one patient each) included acute reversible renal failure following initial cisplatin administration; severe allergic reaction attributed to cisplatin; fatal *Aspergillus* pneumonia (in a complete responder); and generalized exfoliative dermatitis secondary to bleomycin.

TABLE 1.—Patient characteristics

Characteristic	
No. of patients evaluable/entered (%)	28/30 (93)
Mean age in yrs	48.5
Median Karnofsky status, %	75
Prior therapy*	
Irradiation only	15 (53)
Surgery only	2 (7)
Irradiation and surgery	10 (36)
None	1 (4)
Chemotherapy cycles received	
Median	2
Range	0.5–3.5
Overall response rate†	
Pelvic tumor only	1/11 (9)
Pelvic and extrapelvic tumor	4/14 (29)
Extrapelvic tumor only	1/3 (33)
All patients	6/28 (21)
Response duration in mos	3–7+

*Values = No. of patients (%).
†Values = No. of responses/No. of patients (%).

No significant pulmonary toxicity was encountered. Four patients received the maximum cumulative bleomycin dose. In ten patients, carbon monoxide diffusion lung capacity was measured both prior to treatment initiation and following chemotherapy administration. Mean initial and follow-up carbon monoxide diffusion lung capacity values were 87% and 62% of predicted, respectively (mean bleomycin administration, 166 units/m^2). No patient experienced symptomatic pulmonary toxicity. Five patients received a total of 34 days (11 courses) of bleomycin via the Travenol Infusor pumps without medical complications.

DISCUSSION

This study was designed to test the combined activity of bleomycin, mitomycin, and cisplatin in the treatment of advanced cervical cancer. The overall response rate of 21% reported in this study is similar to a previous study of the NCOG (2). In that study, four of 18 patients (22%) experienced partial response, with a median duration of 3 months following treatment with bleomycin and mitomycin. Thus, the addition of cisplatin to these two drugs provided no significant improvement in patient outcome. Failure to achieve a higher response rate may be due to several factors: (*a*) intrinsic tumor insensitivity to the drugs employed; (*b*) frequent dose reductions due to chemotherapy-induced myelosuppression (presumably potentiated by prior pelvic irradiation); and (*c*) suboptimal drug delivery to pelvic disease sites (as suggested by the differential response rates of extrapelvic vs pelvic disease).

No significant pulmonary toxicity was encountered in the current study compared with the 11% rate (two of 18 patients) of serious pulmonary toxicity in our previous study (2). These numbers are too small to draw conclusions regarding bleomycin scheduling and lung toxicity.

Other investigators have tested the drugs utilized in this study for their activity in advanced cervical cancer. Response rates for the bleomycin plus mitomycin drug combination range from 93% in the initial report by Miyamoto et al (6) to 16% reported by Leichmann et al (7). Overall experience with this regimen has shown that responses tend to occur in extrapelvic sites and produce little benefit with respect to overall patient survival (8).

Cisplatin has been combined with bleomycin, mitomycin, and vincristine in two studies. Vogl et al (9) reported that ten of 13 evaluable patients achieved remissions including three complete responses, but median response duration was only 4 months. In a Southwest Oncology

Group study utilizing these four drugs (10,11), a response rate of 47% was reported in 17 patients with recurrent disease, all of whom had received prior pelvic irradiation. Mean survival was only 11 months. The differences in response rates observed in our series in comparison with those cited above are probably not significant. None of these studies has made a major impact on disease-free survival and overall survival in patients with advanced cervix cancer.

In summary, the drug combination of bleomycin, mitomycin, and cisplatin had minimal activity in recurrent and metastatic squamous carcinoma of the uterine cervix. Development of new active agents remains a primary objective in the chemotherapeutic approach to this disease.

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Increased Cytosol Glucocorticoid Receptor Content of Leiomyosarcoma[1,2]

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Glucocorticoid receptor content was determined in cytosol preparations of soft tissue tumors from 42 patients. Leiomyosarcoma had significantly elevated receptors in comparison to malignant melanoma, liposarcoma, malignant fibrous histiocytoma, and invasive desmoid. [Cancer Treat Rep 69:907–908, 1985]

Soft tissue sarcomas are generally not regarded as being responsive to glucocorticoid administration, although this point has probably not been adequately studied. It has previously been observed (1) that certain patients with these tumors, in particular with leiomyosarcoma, will show disease stabilization or regression when treated with prednimustine. Our interest in this observation led to the present study of cytosol glucocorticoid receptor content of several soft tissue tumors.

MATERIALS AND METHODS

Tumor samples were frozen in liquid nitrogen. Portions were submitted for pathological examination for assurance of histological homogeneity. The frozen tumor was stored at -80°C.

Experimental procedures were carried out at 0°C–4°C. Tumor was homogenized in 3 volumes (wt/vol) of TDSM buffer (10 mM tris, 10 mM dithiothreitol, 0.25 M sucrose, and 10 mM sodium molybdate; pH 7.4). The homogenate was centrifuged at 105,000 × *g* for 47 minutes and the supernatant was treated for 2–3 minutes with 10% by volume of dextran-activated charcoal in TDSM buffer [0.5% dextran (wt/vol) and 5% activated charcoal (wt/vol) in buffer]. The mixture was then centrifuged at 1000 × *g* for 7 minutes. The supernatant was utilized for the receptor determination.

Endogenous receptor-bound glucocorticoid was removed from the cytosol preparation with a modification of the method of Banerji and Kalimi (2). Steroid may be dissociated from disulfide bonds on the receptor by treatment with *p*-hydroxymercuribenzoate (PHMB) (3–5). Dithiothreitol (DTT) will reconstitute the original binding capacity of the cytosol after PHMB treatment. For receptor analysis, 10 mM PHMB was prepared in TDSM buffer, 1 volume of this solution was added to 3 volumes of cytosol, and this mixture was incubated for 90 minutes. Charcoal-dextran solution was added (50% by volume), mixed actively for 10 seconds, and then centrifuged twice for 7 minutes at 1000 × *g* to remove the charcoal-dextran with any dissociated steroid.

To each of five tubes, 200 μl of PHMB-treated cytosol, 98 μl of 63 mM DTT in TDSM buffer, and 2 μl of labeled triamcinolone acetonide (TA) were added. In four tubes the final concentration of ^{3}H-TA ranged from 5 to 30 nM, and in the fifth was about 80 nM. In addition, duplicate tubes contained a 100-fold excess of nonlabeled TA. The samples were then incubated for 18 hours.

Following incubation, an aliquot was taken for radioactivity determination, each sample was treated with charcoal-dextran 25% by volume, and the charcoal-dextran was removed by centrifugation. Aliquots were then taken for protein analysis by the method of Lowry et al (6).

This cytosol preparation (250 μl) was assayed for glucocorticoid receptor-^{3}H-TA complex by isoelectric focusing (7). After a 1.5-hour run, the gel from each sample was cut into strips, which were counted. The peak radioactive area was observed at an approximate gel pH of 5.2. This peak always disappeared when an excess of unlabeled TA was added to the radioactive TA, indicating a cytosol protein of high-binding affinity and low-binding capacity.

The "specific" count in the glucocorticoid receptor area for each sample was obtained by adding the

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counts in the 2-cm gel strip centering at a pH of 5.2 and subtracting from this value an estimate of the background count of the gel derived from the count on either side of this peak area. The binding site dissociation constant (K_d) and the total binding capacity were calculated using Scatchard plot analysis (8). The tumor curves showed evidence of only a single receptor binding site.

RESULTS

Data from tumor specimens from 42 patients are presented in table 1. One of the specimens from patients with malignant melanoma showed a fourfold increase in concentration of binding sites when compared to the other melanoma specimens, and this value is presented separately. The leiomyosarcoma specimens were observed to have increased binding site content. When compared to the 12 melanoma values, the leiomyosarcoma values were significantly elevated ($P < 0.001$). They were also significantly greater than desmoid ($P < 0.001$), fibrous histiocytoma ($P < 0.001$), and liposarcoma ($P < 0.001$) values.

TABLE 1.—Glucocorticoid receptor binding capacity and affinity in cytosol preparations of human soft tissue tumors

Tumor*	Concentration of binding sites (fmols/mg of protein)†	$10^{-9} \times K_d$†
Leiomyosarcoma (8)	172 ± 45	6.3 ± 2.2
Malignant melanoma (12)	61 × 43‡	6.7 ± 3.9
Malignant melanoma (1)	251	8.6
Liposarcoma (5)	31 ± 25‡	4.8 ± 1.6
Malignant fibrous histiocytoma (4)	36 ± 21‡	4.9 ± 1.0
Desmoid (4)	52 ± 28‡	5.7 ± 2.2
Chondrosarcoma (2)	39, 21	3.3, 7.1
Mesothelioma (2)	35, 77	5.1, 4.7
Hemangiopericytoma (1)	252	5.1
Malignant lymphoma (1)	247	10.2
Embryonal rhabdomyosarcoma (1)	71	8.3
Neurogenic sarcoma (1)	47	2.8

*No. of tumor specimens, each from a different patient, is given in parentheses.

†Values = mean ± SD.

‡Each of these binding site values differs significantly from the leiomyosarcoma value ($P < 0.001$). Results were analyzed by Student's t test.

A solitary, subcutaneous mass of malignant lymphoma, in addition to a single specimen of a hemangiopericytoma, showed high concentrations of glucocorticoid binding sites.

The binding site dissociation constant values for these tumors are also shown in table 1; they could not be shown to differ significantly from one tumor type to another.

DISCUSSION

The leiomyosarcoma is not known to be a glucocorticoid-sensitive tumor. The present study has demonstrated a significantly increased concentration of glucocorticoid receptors in leiomyosarcoma in comparison to malignant melanoma, desmoid, malignant fibrous histiocytoma, and liposarcoma. It has been previously reported (1) that leiomyosarcoma frequently showed response or stability of disease following prednimustine administration. If leiomyosarcoma is not a steroid-sensitive tumor, the question arises as to whether a high concentration of steroid receptor may attract increased quantities of this prednisolone-chlorambucil ester to the tumor and thereby increase the therapeutic index of the chlorambucil moiety.

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Evaluation of a Combination Antiemetic Regimen Including Iv High-Dose Metoclopramide, Dexamethasone, and Diphenhydramine in Cisplatin-Based Chemotherapy Regimens[1]

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The control of emesis in cancer patients receiving chemotherapy has proved to be important in support management. Metoclopramide (MCP), a substituted benzamide, has been widely used, and preclinical trials in dogs and ferrets have indicated excellent activity against emesis by cisplatin (DDP) when MCP was given in high doses (1). Several phase II studies have shown a high grade of efficacy with high-dose MCP given iv (2,3). The suggestion that the dose and schedule of MCP are important if an antiemetic effect is to be observed prompted us to further test the results of other investigators who found that shorter courses of MCP at higher doses in combination with dexamethasone (DXM) and diphenhydramine (DPH) improved the antiemetic response without toxicity (4).

PATIENTS AND METHODS

Eligibility requirements included histologic confirmation of malignancy and progressive metastatic disease without prior chemotherapy. Patients were required to have a wbc count $> 4000/mm^3$, platelet count $> 120,000/mm^3$, creatinine clearance > 65 ml/minute, and total bilirubin < 2 mg/dl. The study was designed to accrue 30 patients undergoing treatment with DDP combination chemotherapy, using iv high-dose MCP plus DXM and DPH as an antiemetic regimen. Twenty-two patients received DDP (60 mg/m^2), doxorubicin (40 mg/m^2), and cyclophosphamide (400 mg/m^2) for lung and ovarian cancer, and eight received DDP (100 mg/m^2) plus 5-FU (1000 mg/m^2/day × 5 by continuous infusion) for head and neck cancer. All patients were hospitalized for their first three courses.

MCP (3 mg/kg of body weight) in 250 ml of 0.9% sodium chloride solution was infused for 30 minutes before the 30-minute infusion of DDP and then 90 minutes later. DXM (20 mg) was infused in a 100-ml aliquot 10 minutes prior to the first MCP dose. Also, DPH (50 mg) was given orally. DXM (20 mg) and DPH (50 mg) were administered once only.

RESULTS

As shown in table 1, the mean number of emeses per

TABLE 1.—Patient characteristics and treatment results

Characteristic	No. of patients (%)
Total	30
Age (yrs)	
Median: 61	
Range: 28–76	
Sex	
Men	23
Women	7
Performance status (%)	
80–90	18
60–70	12
Diagnosis	
Lung cancer	18
Ovarian cancer	4
Head and neck cancer	8
Chemotherapy regimens	
DDP, doxorubicin, and cyclophosphamide	22
DDP and 5-FU	8
No. of chemotherapy courses	
1	12
2	5
3	13
No. of emetic episodes during 1st course	
Median: 0.5	
Range: 0–4	
0–2 major antiemetic responses	25 (83.3)
Other toxic effects	
Mild sedation	13 (43)
Diarrhea	5 (16.6)
Acute dystonic reactions	0

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patient on the first course was 0.5 (range, 0–4). There were 23 men and seven women, with a median age of 61 years (range, 28–76) and a median performance status of 80%. Eighteen patients had lung cancer, four had ovarian cancer, and eight had head and neck cancer. Twelve patients received one course, five received two, and 13 received three. During the 24-hour observation period of 61 evaluable courses, 60.6% of the patients had no nausea or vomiting and 75.4% had no vomiting in any course. When present, nausea was mild and the median number of vomiting episodes was 0.7 (range, 0–9). Only five of the 30 patients had late occurrence of nausea and vomiting of moderate intensity, which did not require the administration of a third high dose of MCP.

In addition, MCP was well-tolerated with minimal toxicity. Mild sedation occurred in 48% of the patients and 16.6% had diarrhea (three or more loose bowel movements). Acute dystonic reactions were not observed.

DISCUSSION

Our results are similar to those of other studies in which an antiemetic regimen with iv high-dose MCP produced major antiemetic responses (0–2) in 70% of patients, with 61% having no emesis (4). We conclude that the time frame used for the administration of MCP to encompass the peak period of emesis induced by moderate (60 mg/m^2) and high-dose (100 mg/m^2) DDP regimens is sufficient and that this combination antiemetic regimen is a highly effective prophylaxis for nausea and vomiting without significant side effects.

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Evaluation of Cisplatin in Children With Recurrent Brain Tumors[1,2]

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Cisplatin (CDDP) is a cycle-nonspecific agent which has neural toxicity and pharmacologic behavior that suggests CNS penetration (1). A therapeutic benefit of CDDP for patients with recurrent brain tumors has been reported (2–4). Here we report a phase II trial in 14 children with recurrent brain tumors.

MATERIALS AND METHODS

Children who had relapsed from prior surgery, chemotherapy, and/or radiotherapy were eligible for treatment with CDDP. The schedule used was 60 mg/m^2 given as a 4-hour infusion for 2 consecutive days every 4 weeks. No patients received corticosteroids. Responses were evaluated by physical examination and computerized tomographic scan. Toxic effects were evaluated by published standards (5).

RESULTS

Fourteen patients received 47 courses (median, three). Two patients were not evaluable due to early death (18 and 48 hours after the first course of CDDP). The diagnoses and responses are shown in table 1. No complete or partial responses were seen. Four of 12 patients had stable disease. Two of these patients showed slight clinical improvement without change in the computerized tomographic scan (Patient 3) or a < 50% change (Patient 10). Only one patient with stable disease continued in this status for 33+ months; the other showed progressive disease after 4–6 and 17 months. Complete toxicity data are shown in table 2.

Our experience with CDDP in children with recurrent brain tumors has been disappointing. We do not confirm the experience of others.

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TABLE 1.—Patient characteristics at treatment start and responses*

Patient No.	Age (yrs)/ sex	Histologic diagnosis	Initial therapy			Disease recurrence (mos)/site	Therapy for recurrence prior to CDDP	Time from last therapy to start of CDDP (mos)	No. of CDDP courses	Response/ duration (mos)‡
			Surgery	Radiotherapy	Chemotherapy†					
1	9/M	Medulloblastoma posterior fossa	90%	6250 rad posterior fossa 3000 rad craniospinal	—	39/temporal lobe	None	37	3	PD/—
2	2/F	Medulloblastoma parietal	Partial	5600 rad parietal 3600 rad craniospinal	MTX + BCNU	6/local	None	4	3	PD/—
3	5/F	Medulloblastoma posterior fossa	Total + VP	5600 rad posterior fossa 3600 rad craniospinal	VCR + CCNU	36/supratentorial	None	28	5	SD/17
4	11/M	Medulloblastoma posterior fossa	VP + biopsy	5600 rad posterior fossa 3600 rad craniospinal	—	20/local supratentorial	None	18	2	SD/4
5	14/F	Medulloblastoma posterior fossa	90%	6430 rad posterior fossa 2550 rad craniospinal	—	29/local	None	23	3	PD/—
6	6/F	Medulloblastoma posterior fossa	Partial	5600 rad posterior fossa 3600 rad craniospinal	MTX + BCNU	22/CSF supratentorial	None	5	1	PD/—
7	8/M	Astrocytoma IV parietal	Partial	4420 rad	—	4/local	None	3	2	PD/—
8	8/M	Astrocytoma I optic	—	3500 rad	—	48/local	3000 rad + VP			
9	15/M	Astrocytoma I posterior fossa	VP + partial	5600 rad posterior fossa 2000 rad craniospinal	—	56/local 68/local	Partial surgery None	12	8	SD/33+
10	5/M	Brain stem glioma	—	4950 rad	—	11/local	Partial surgery	2	3	PD/—
11	9/F	Chordoma	—	4020 rad	—	12/local	None	10	7	SD/6
12	2/M	Choroid plexus carcinoma	90%	4000 rad	—	24/local 8/local	None None	24 6	5 2	PD/— PD/—

* VP = ventricle peritoneal shunt.
†MTX = methotrexate; BCNU = carmustine; VCR = vincristine; and CCNU = lomustine.
‡PD = progressive disease; SD = stable disease.

TABLE 2.—Toxic effects

	No. of patients			
	Toxicity grade—			
	0	1	2	3
Hematologic*				
Hemoglobin	4	3	3	2†
Wbc	11	1	0	0
Platelet	11	1	0	0
Renal*				
BUN	3	5	1	3
Creatinine clearance	9	0	1	2
Gastrointestinal*				
Nausea/vomiting	0	0	13	0
Hepatic (GOT/GPT)	10	2	0	0
CNS‡				
Hearing			2§	
Neurologic	11	0	0	1
Metabolic‡	9	1	1	1

* *See* ref 5.
† Patients 2 and 3.
‡ *See* ref 2.
§ Only these 2 patients had serial hearing tests.

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Phase II Trial of 4-Demethoxydaunorubicin in Patients With Advanced Malignant Melanoma[1,2]

Gail Stanton, Ephraim S. Casper,* and Barbara Friedman[3]

Current chemotherapy for malignant melanoma is inadequate and there is a critical need for new active drugs. Anthracyclines have shown definite, albeit limited, activity in the treatment of this disease (1,2). 4-Demethoxydaunorubicin (DMDR) is a new anthracycline derivative that showed a broad spectrum of antitumor activity in preclinical testing (3) and was well-tolerated in a phase I clinical trial (4). We therefore conducted this phase II trial of DMDR in patients with advanced malignant melanoma.

PATIENTS AND METHODS

All patients in this study had a pathologically confirmed diagnosis of malignant melanoma. Additional eligibility criteria included the presence of measurable lesions, performance status of at least 50% (Karnofsky scale), normal hepatic and renal functions, wbc count $\geq 4000/mm^3$, and platelet count $\geq 100,000/mm^3$.

DMDR was supplied by Farmitalia Carlo Erba. The initial dose was 12.5 mg/m^2 iv every 3 weeks. However, this dose was nonmyelosuppressive in many patients, and, as the study progressed, the starting dose was increased to 15 mg/m^2. Doses were modified on the basis of nadir blood cell counts. An attempt was made to treat patients with at least two doses of the drug; however, if after the first dose there was unequivocal progression of disease, that patient's trial was considered adequate and therapy with DMDR was stopped. Standard criteria for therapeutic response were employed.

RESULTS

Fifteen patients were entered in this protocol, 14 of whom are evaluable for therapeutic response. One previously untreated patient died of progressive liver involvement 11 days after receiving her first dose of DMDR. The characteristics of the 14 evaluable patients are presented in table 1. Ten patients received two or more doses of the drug, and four received only one dose because of rapid progression of disease. Two patients had stable disease for 3 and 5 months, respectively; all others had clear progression of disease within 2 months of starting treatment.

Leukopenia was the major toxic effect. The median wbc count nadir was 2.9 × $10^3/mm^3$ (range, 0.4-9.7); the median platelet count nadir was 191.0 × $10^3/mm^3$ (range, 109.0-601.0). The nadir count was usually documented on Day 15 after drug administration. All patients had progressive anemia, which may have been related to progressive disease or to treatment with DMDR; the median

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 54 (22-69)	
Sex	
Male	12
Female	2
Median performance status (range): 80% (60%-90%)	
Prior chemotherapy	
Yes	3
No	11
Predominant site of disease	
Skin, nodes	10
Lung	1
Liver, gastrointestinal tract	3
Wbc count nadir (× $10^3/mm^3$)	
≥ 4.0	3
3.0-3.9	3
2.0-2.9	7
< 2.0	1
Platelet count nadir (× $10^3/mm^3$)	
≥ 150.0	11
100.0-149.0	3

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fall in hemoglobin from baseline was 2.4 g/dl (range, 0.9–7.4). Mild nausea or vomiting was observed in ten patients, and four had mild stomatitis. There was no alopecia or any evidence of drug-related hepatic, renal, or cardiac dysfunction.

DISCUSSION

The patients in this study had good performance status and most had not received prior systemic chemotherapy; most had skin or lymph nodes as their major site of disease. Nonetheless, objective tumor regressions were not observed. The true response rate, therefore, is $< 20\%$ ($P < 0.05$). We conclude that when used iv, DMDR lacks sufficient activity in the treatment of advanced malignant melanoma to warrant further study at the dose and schedule tested.

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Phase II Evaluation of Melphalan in Adenocarcinoma of the Pancreas[1,2]

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For the North-Western Gastro-Intestinal Tumour Group

Most patients with carcinoma of the pancreas die within 12 months of diagnosis (1). Despite this, few drugs have been adequately tested in this disease; in particular, there are few data on response to alkylating agents. Melphalan is active against ovarian (2), breast (3), and colon (4) cancers and has been tolerated in doses of up to 125 mg/m^2 in phase I trials (5). This study investigates the activity of moderate-dose melphalan against pancreatic carcinoma.

PATIENTS AND METHODS

Fifteen patients with advanced adenocarcinoma of the pancreas who had received no prior chemotherapy were entered in the study. Disease was measurable clinically in five patients, by ultrasound scan in six, and by computerized tomographic scan in four.

Melphalan (1 mg/kg) was administered in 250 ml of normal saline by short iv infusion and repeated every 28 days. The dose was reduced by 50% if the wbc count nadir was $< 1.0 \times 10^9$/L or the platelet count nadir was $< 50 \times 10^9$/L and was delayed if full hematologic recovery had not occurred when re-treatment was due. Chemotherapy was discontinued if there was documented disease progression after two or more courses.

RESULTS

Patient characteristics are shown in table 1. There were two partial responses (13%), one of 6 weeks' duration and one of 36+ months' duration. Both responses occurred in patients with local disease only and were confirmed by computerized tomographic scan. Myelosuppression resulted in eight (16%) treatment delays and in seven (14%) dose reductions. There was one septicemic death and three additional patients required blood and platelet transfusions (platelets $< 20 \times 10^9$/L) for life-threatening gastrointestinal hemorrhage. All patients experienced severe nausea and vomiting continuing for up to 5 days and followed by anorexia and malaise for a variable period.

TABLE 1.—Patient characteristics

Characteristic	
Age (yrs)	
Median	60
Range	39–72
Sex*	
Male	8
Female	7
Karnofsky performance status (%)	
Median	60
Range	50–90
Wks from diagnosis of primary to 1st infusion	
Median	4
Range	2–32
Prior surgery*	
Bypass	9
Biopsy	6
Sites of disease*	
Local only	12
Local + liver	1
Local + supraclavicular fossa nodes	1
Local + lung + supraclavicular fossa nodes	1
No. of courses	
Median	3
Range	1–6
Survival from 1st infusion (wks)	
Median	16
Range	2–144+

* Values = No. of patients.

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[2]Melphalan was supplied by The Wellcome Foundation Ltd, Beckenham, Kent, England.

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DISCUSSION

The median survival of 16 weeks in this study is little different from that of untreated patients (1). Moreover, in view of the serious nonhematologic toxicity and unpredictable gastrointestinal bleeding, a single durable remission did not justify continuing the trial. The 13% response rate, however, indicates a measure of activity for melphalan against pancreatic carcinoma, and a high-dose regimen with autologous marrow rescue may improve response and survival.

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Phase II Trial of Carboplatin in Advanced Breast Carcinoma: A Cancer and Leukemia Group B Study[1,2]

Bruce W. Booth,[3,4] Raymond B. Weiss,[3,4,*] Ann H. Korzun,[5] William C. Wood,[6] Robert W. Carey,[6] and Lawrence C. Panasci[7,8]

Carboplatin is a second-generation platinum analog synthesized at Johnson Matthey Research Centre in Great Britain. With its relatively stable leaving ligand, carboplatin presumably acts as a "pro drug," first requiring transformation in vivo prior to interacting with the appropriate cellular target (1). Although it results in DNA intrastrand and interstrand cross-linking like cisplatin, carboplatin also causes more nuclear protein phosphorylation in tumor versus normal cells (2,3). This finding correlates with cytotoxicity and suggests some degree of selectivity. In preclinical and phase I studies, carboplatin caused less gastrointestinal and auditory toxicity than cisplatin and had minimal renal toxicity even without hydration (4). Clonogenic assay studies suggested that 24-hour drug exposure increased cytotoxicity (5). Using a maximum tolerated dose for 24-hour infusion determined in a phase I trial (5), we evaluated the activity of carboplatin in advanced breast carcinoma.

MATERIALS AND METHODS

Twenty women with histologically confirmed metastatic adenocarcinoma of the breast were entered in the trial between January and May 1983. Eligibility criteria consisted of the following: measurable disease, performance score ≤ 2, life expectancy > 2 months, prior treatment with no more than two prior chemotherapeutic regimens, > 4 weeks since prior chemotherapy or radiotherapy, no prior treatment with a platinum-containing drug, wbc count $> 3500/\mu l$, platelet count $\geq 100{,}000/\mu l$, hemoglobin > 10 g/dl, creatinine < 1.5 mg/dl, bilirubin < 1.5 mg/dl, creatinine clearance > 60 ml/minute, and informed consent.

Carboplatin was given as a 24-hour infusion by constant-flow pump at a dose of 320 mg/m^2 for good-risk patients (no prior nitrosoureas or mitomycin and irradiation to $\leq 50\%$ of marrow-containing skeleton) or 280 mg/m^2 for poor-risk patients (history of use of nitrosoureas or mitomycin or extensive irradiation). Treatment was repeated every 28 days. Therapy was continued until disease progression; it was given for at least two cycles of treatment unless disease progression was rapid. Standard criteria for measuring tumor response were used.

RESULTS

Of the 20 patients entered, one was never treated, four were ineligible (three had more than two different prior chemotherapy regimens and one had bilirubin of 8.0 mg/dl at the start of treatment), and one had inadequate data to assess either response or toxicity. Patient characteristics and toxicity are shown in tables 1 and 2. All 14 of the evaluable patients had been heavily treated previously; three had had one chemotherapeutic regimen and 11 had had two regimens. The average number of drugs per patient was 6.1. Eleven patients had had prior radiotherapy. Eight patients were treated with the 320-mg/m^2 dose and six were treated with the 280-mg/m^2 dose.

There were no objective responses to carboplatin. Four patients temporarily had stable disease. The median time to treatment failure (the time from entry in the study to the date of disease progression or death if no documented progression) was 9.5 weeks.

Toxicity was relatively low. Dose reduction was required only in one patient in whom the platelet count dropped to 10,000/μl on Day 56; no bleeding occurred. Leukopenia was mild, did not correlate with drug dose level, and was not associated with infection. Carboplatin

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	
Entered	20
Evaluable	14
Median age in yrs (range): 56 (35–76)	
Menopausal status	
Premenopausal	1
Postmenopausal	13
Natural	7
Surgical	4
Chemotherapeutic	2
Estrogen receptor status	
Negative	5
Borderline	2
Positive	5
Unknown	2
Performance score	
0 (normal)	4
1 (ambulatory)	7
2 (< 50% time in bed)	3
Dominant disease site	
Soft tissue	3
Osseous	1
Visceral	10
Prior therapy	
Hormonal therapy	9
Radiotherapy	11
Chemotherapy	14
1 regimen	3
2 regimens	11

TABLE 2.—Toxicity

Toxic effect	No. of patients
Nausea, vomiting	9*
Leukopenia (wbcs/mm^3)	
3000–3999	6
Thrombocytopenia (platelets/mm^3)	
100,000–149,000	6
75,000–99,999	2
25,000–74,999	3
< 20,000	1
Hypersensitivity reaction	1
Nephrotoxicity	0

* 1 mild, 7 moderate, and 1 severe.

caused less nausea and vomiting than cisplatin, and iv fluid replacement was not required. Occasionally, vomiting without nausea occurred. No nephrotoxicity was observed. One patient had urticaria and fever indicative of a type I hypersensitivity reaction following both of her infusions; these symptoms responded to antihistamine treatment.

DISCUSSION

Cisplatin is not particularly effective in breast cancer, analogs would allow higher drug concentration at the cellular level, with consequent increased therapeutic activity. However, in this phase II trial no response was seen with carboplatin. Most of these patients had visceral-dominant disease and had received two prior chemotherapy regimens. Carboplatin toxicity was relatively low, and nephrotoxicity was not seen.

In conclusion, although relatively well-tolerated, carboplatin in this dose and schedule is not active in this pretreated population of patients with advanced breast cancer.

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Phase II Clinical Trial of Diaziquone in Bronchogenic Carcinoma[1,2]

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Diaziquone (AZQ), one of a series of quinone derivatives demonstrating a broad-spectrum antitumor activity against transplantable murine tumor systems (1), is of interest because of its unique lipophilic, low ionization, and water solubility properties that allow it to penetrate into cerebrospinal fluid (2). Clinical trials with AZQ demonstrate antineoplastic activity in brain tumors (3). Because of frequent brain metastasis and the limitations of standard treatments in bronchogenic carcinoma, newer agents are being tested. Here we report a phase II study of AZQ in advanced cases of bronchogenic carcinoma, using a previously determined dose schedule (4).

PATIENTS AND METHODS

Our study evaluated 40 patients with locally advanced or metastatic bronchogenic carcinoma (table 1); those with brain metastasis had received cranial irradiation. All patients had stage III disease, with extensive disease in 37. Metastatic sites included brain (26 patients), bone (15 patients), liver (nine), lung (seven), nodes (six), adrenals (three), bone marrow (two), and skin (one). AZQ, obtained from the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), was injected iv daily for 5 days at doses of 6 mg/m^2 for poor-risk (age $\geq$ 70 years, debilitated, prior chemotherapy) and 8 mg/m^2 for good-risk (age $<$ 70 years, performance status $\leq$ 2, no prior chemotherapy) patients. Treatment was repeated at 4-week intervals, with a median of two courses (range, one to eight). Physical examinations, routine tests (such as those for hematologic and biochemical parameters), and chest roentgenographs were done before treatment and during each course. Standard response criteria were used.

RESULTS

Of the 40 patients, 11 had no change in lesion size after AZQ, while 26 had increasing disease; median time to disease progression in nonresponders was 5 weeks (range, 4-16). In the remaining three patients, all of whom had disease in the lung and single-site metastasis in the brain, there were two partial responses (PRs) and one minor response. Lung lesion regressions lasted 11-16 weeks. All PRs were in previously untreated patients who had received cranial irradiation. Responses were also seen in

TABLE 1.—Patient characteristics

Characteristics	No. of patients
Total evaluated	40
Median age in yrs (range): 54 (33-76)	
Performance status (Zubrod scale)	
0	3
1	12
2	9
3	6
4	4
Unknown	6
Prior treatment	
Chemotherapy alone	10
Radiotherapy + chemotherapy	11
No prior treatment	19
Tumor type	
Adenocarcinoma	23
Squamous cell carcinoma	7
Poorly differentiated carcinoma	5
Large cell carcinoma	2
Type unknown (non-small cell)	2
Small cell carcinoma	1

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brain lesions. The PR rate was 11% of the 19 previously untreated patients and 5% of all patients, with no complete responses.

Myelosuppression was the dose-limiting toxic effect (table 2). Two patients had gastrointestinal bleeding, one had epistaxis, and one had gum bleeding and vaginal spotting. Neutropenia was associated with fever in 8% of courses and with documented infection in 3%. Table 2 summarizes other toxic effects.

TABLE 2.—Toxicity

Toxic effect	% of courses
Neutropenia (< 1000 cells/mm^3)	17
Thrombocytopenia (< 50,000 cells/mm^3)	16
Nausea/vomiting	12
Anorexia	5
Diarrhea	3
Mucositis with dysphagia	1

DISCUSSION

Our 5% PR rate corresponds to that reported by investigators using AZQ at doses of 30–35 mg/m^2 every 3 weeks in previously untreated patients with non-small cell bronchogenic carcinoma (5). Because of this limited activity, further studies of AZQ as a single agent in non-small cell bronchogenic carcinoma do not seem warranted.

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Treatment of Blast Crisis of Chronic Myelogenous Leukemia With Vincristine, Prednisone, and Cytarabine

The terminal period of chronic myelogenous leukemia (CML) is manifested in most instances by two different hematologic patterns, ie, an accelerated myeloproliferative phase and a blastic phase very similar to acute leukemia. Agents employed in the treatment of acute myeloblastic leukemia are generally ineffective in blast crisis of CML (1). On the other hand, several reports have suggested that patients in blast crisis with lymphoblastic morphology and markers may have an improved response rate following therapeutic regimens active in acute lymphoblastic leukemia, especially the combination vincristine-prednisone (2,3).

PATIENTS, MATERIALS, AND METHODS

In an attempt to encompass both main cytological types of blast crisis of CML (ie, lymphoid and myeloid), between January 1979 and November 1983, 22 consecutive such patients were given courses of vincristine (total dose, 2 mg iv on Day 1), prednisone (100 mg/m^2 iv on Days 1-6), and cytarabine (100 mg/m^2 of continuous iv infusion on Days 2-6) as first therapeutic regimen. In 21 patients, CML had been diagnosed in the chronic phase of the disease, whereas one patient presented with an extramedullary (lymph node) blast crisis of Ph^1-positive CML. Cytogenetic studies were available in 20 patients, and the Ph^1 chromosome was found in 18. There were 12 males and ten females, with a mean age of 40.9 years (SD ± 15.7). The diagnosis of blast crisis was based on standard criteria (2). The cytological type of blast crisis (lymphoid or myeloid) was assessed by examination of the morphology and cytochemical reactions (peroxidase and periodic acid-Schiff) of the blast cells. Terminal deoxyribonucleotidyl transferase (TdT) was investigated in only three cases.

Complete remission was defined by the following criteria: (*a*) normal wbc counts (without blast cells) and platelet counts > 100 × 10^9/L; (*b*) blast cells < 5% in a cellular bone marrow aspirate; and (*c*) disappearance of all signs of extramedullary disease if present prior to therapy. Partial remission was defined by the following criteria: (*a*) substantial increase in platelet and wbc counts (without blast cells); (*b*) disappearance or a clear-cut reduction of the signs of extramedullary disease observed before the institution of therapy; and (*c*) reduction in bone marrow blast cells to < 20% or reduction in bone marrow blast cells plus promyelocytes to < 50% of the initial value. Patients who did not meet the criteria of these two categories after a minimum of three courses of induction therapy were considered nonresponders. Responding patients received two additional courses of the induction regimen as consolidation therapy; subsequently, maintenance therapy was instituted with either mercaptopurine or hydroxyurea at a dosage adjusted for changes in the wbc and platelet counts. Actuarial survival curves were plotted according to the method of Kaplan and Meier, and were statistically compared using the log-rank test. Pretreatment characteristics of responders and nonresponders were compared by means of the χ^2 test with Yates' correction and Student's t test.

RESULTS AND DISCUSSION

A total of 50 courses of induction therapy were administered. In all cases, cytolytic effect was observed, although it was transitory in some of them. Five complete and one partial remission were obtained, for an overall remission rate of 27%. In five of the six responders, remission was achieved following the first chemotherapy course. At this time, 20 patients have died due to infection (15 patients) or hemorrhagic complications (five). Median survival for the entire group of patients was 86.2 days (range, 17-656) from the start of therapy. Median survival for responders was 201 days, versus 65 days for nonresponders, with the comparison of both survival curves approaching significance ($P = 0.058$). No differences were found between pretreatment characteristics of responders and nonresponders (table 1), except for the incidence of older age in the former group of patients ($P < 0.01$). The most frequent and life-threatening adverse effect of therapy was severe and prolonged marrow aplasia, which was observed in 13 patients after 20 courses of induction therapy and was often complicated by infection and hemorrhage.

In confirmation of the known poor therapeutic responsiveness of blast crisis of CML (4), the above results do not differ from those reported by other investigators with the combination vincristine-prednisone. The low proportion of patients with lymphoid type of blast crisis observed in the present series did not allow us to evaluate the therapeutic response in respect to the cytological type of the blast cells. As established in previous reports

TABLE 1.—Pretreatment characteristics of responders and nonresponders*

	Responders	Nonresponders
Sex		
Males	2	10
Females	4	6
Age (yrs)		
Mean (SD)	54.6 (12)	35.7 (13.9)
Time from diagnosis of CML to blast crisis (mos)		
Mean (SD)	25.8 (18.9)	40.3 (32.5)
Total busulfan dose (mg)		
Mean (SD)	643 (467)	938 (707)
Spleen		
Not palpable or < 5 cm†	4	5
Palpable ≥ 5 cm†	2	11
Extramedullary blastic involvement	1	4
Wbc count (× 1000/mm³)		
Mean (SD)	54 (66.7)	91.1 (118)
Platelet count (× 1000/mm³)		
Mean (SD)	324 (413)	180 (144)
Blast cell phenotypic type		
Myeloid	5	15
Lymphoid	1	1

* Unless specified otherwise, values = No. of patients.
†Below the left costal margin.

(4), responding patients had a significant prolongation of survival. Interestingly, most of the responders achieved the remission after a single course of therapy, supporting the suggestion that in blast crisis the therapeutic responsiveness seems to depend on the therapeutic sensitivity of the blast cells rather than on increasing number and combinations of drugs (5). Finally, analysis of a greater number of patients is required to ascertain the prognostic significance of parameters present at diagnosis of blast crisis.

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Difficulty of Interpreting Disease-Free Interval Curves When Substantial Numbers of Patients Die in Remission

The method most commonly used to calculate the probability of survival and duration remission in patients with leukemia is the Kaplan-Meier product-limit estimation. However, there is no unanimous agreement as to when data should be considered censored or uncensored, specifically when a death in complete remission occurs. In evaluating overall survival, all deaths are generally counted as events; therefore, survival times for such patients are considered uncensored. However, for computing disease-free interval curves (DFIC), there are differences of criteria in statistically treating the patients who die while in remission. The problem is that death during remission is a risk that competes with the risk of relapse. Most papers describe the patient as withdrawn alive (censored) at the time of death, which is essentially the Kaplan-Meier procedure. Recently, some authors interpreted death in remission as relapse for statistical purposes (1-3), assuming that the patient who dies would have had a relapse in the very next instant. A third alternative, not usually used, would be to assume that those who die would never have developed a relapse. These last two alternatives yield a lower and upper bound, respectively, on the DFIC (4).

The current difference of criteria to calculate DFIC can lead to considerable confusion, especially with the increasing aggressiveness of the maintenance protocols which involve a high risk of death to patients in remission. An example is our series of 26 patients with acute nonlymphocytic leukemia in complete remission who had a mortality of 30.8% (eight cases) as a consequence of therapy-induced complications (5). When calculating the actuarial curve of remission duration (fig 1), an impor-

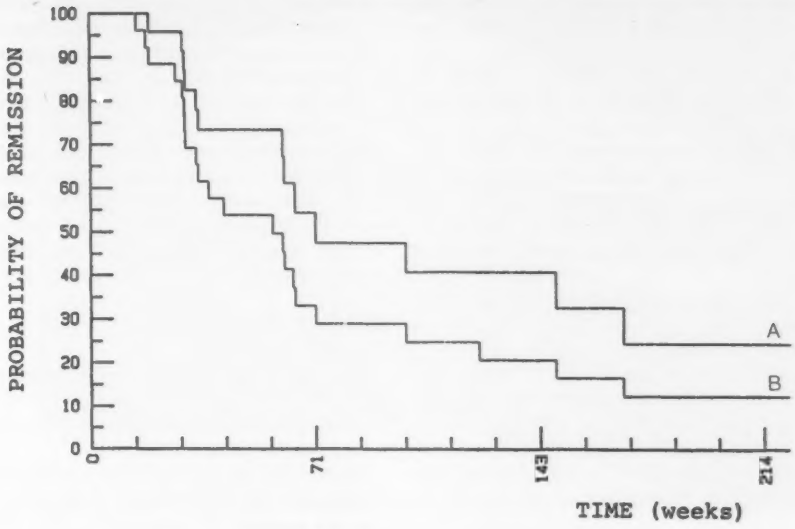

FIGURE 1.—A = death in remission as censored data; B = death in remission as uncensored data.

tant variation is observed, depending on the criteria used.

In summary, we believe that until a solution is given for solving the problem of the interpretation of DFIC when a substantial number of patients die while in remission, the criteria applied to decide whether data are considered censored or uncensored should be clearly shown in the methods.

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Extensive Tissue Ulceration Due to Apparent Sensitivity Reactions to Mitomycin

Extreme local tissue ulceration results in inadvertent drug extravasation of mitomycin. The clinical sequelae most resemble the indolent characteristics of doxorubicin-induced ulcerations, which often require wide excision (1).

The literature reveals several reports of tissue toxicity in circumstances where it would not be anticipated (2-4). Similarly, the following case reports describe unusual reactions where generalized tissue necrosis involving the entire forearm(s) resulted after the *second* treatment of mitomycin but which had been preceded by generalized, yet painless venous hyperpigmentation after *first* exposure.

CASE REPORTS

Patient 1

A 47-year-old white male with a history of poorly dif-

ferentiated adenocarcinoma of the lung, initially treated with doxorubicin and later with semustine and 5-FU, was evaluated for further treatment with mitomycin.

The first infusion of mitomycin (40 mg), given by iv push, was performed without incident. Generalized venous hyperpigmentation developed along the entire forearm used for his original treatment without accompanying discomfort. In an attempt to prevent further venous changes, the second dose (40 mg) was delivered into the injection port of a rapidly infusing iv line in the contralateral arm. The patient tolerated the second treatment without incident.

Within 7 days after the second treatment, numerous ulcerations appeared along the entire course of the right forearm vein where the second dose had been delivered. In addition, ulcerations were noted on the dorsum of the left hand in the area of his first treatment. No further treatments were given.

Four months after the initial treatment, the patient had further tissue breakdown on the right forearm, requiring multiple plastic surgery interventions.

Patient 2

A 73-year-old white male with a diagnosis of poorly differentiated adenocarcinoma of the stomach was considered for adjuvant chemotherapy consisting of 5-FU, doxorubicin, and mitomycin (FAM). However, due to the patient's cardiac history, it was decided to omit doxorubicin.

At the first treatment, the patient received 5-FU (1000 mg) given by iv push, followed by mitomycin (15 mg) given through the injection port of a rapidly infusing iv line in the left arm. Within 7 days, generalized venous hyperpigmentation was noted involving the entire arm used for treatment (fig 1).

Two months later, the contralateral forearm was used for administration of a second dose of mitomycin (15 mg). To prevent further venous changes, the drug was diluted in 150 ml of normal saline and infused without incident. Within 4 days, several ulcerated areas had formed on the right forearm several inches proximal to the second injection site (fig 2). As with the first patient, plastic surgery interventions were required to complete healing.

DISCUSSION

An explanation for the aforementioned phenomenon is not the intention of this article. However, the similarity of these two cases should serve to alert other clinicians of this potential tissue reaction.

A report of generalized venous hyperpigmentation following the administration of single-agent 5-FU without morbid sequelae (5) may explain the venous hyperpigmentation, but does not account for the extensive tissue necrosis reported here. While only mitomycin is considered to have vesicant properties, both drugs have been reported to produce tissue changes after exposure to sunlight (4,5). This could explain the occurrence of tissue necrosis as a later phenomenon if subclinical venous irritation occurred initially.

While it is unclear whether cessation of mitomycin after occurrence of generalized venous hyperpigmentation would prevent subsequent ulceration, or if protection

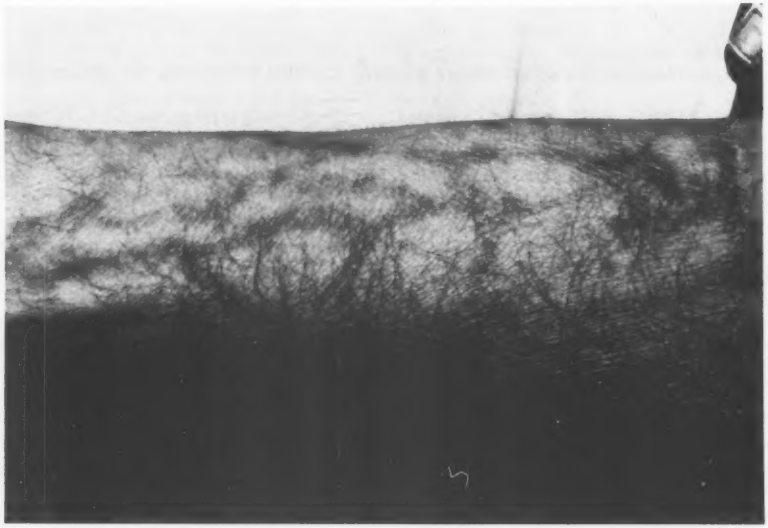

FIGURE 1.—Painless venous tracking reported 7 days after the 1st treatment with mitomycin.

FIGURE 2.—Extensive tissue ulceration 4 days after the 2nd injection of mitomycin had been infused without incident.

from exposure to sunlight would do the same, we recommend careful consideration of the possibility that a relationship may exist between venous hyperpigmentation and subsequent ulceration with this drug combination.

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Chemotherapy in Advanced Gastric Cancer

In gastric cancer, Klein et al (1) recently reported a high response rate (63%) to a combination of three cytotoxic drugs, and emphasized the lack of toxicity. The regimen consisted of the following: Day 1, methotrexate (1.5 g/m^2 iv bolus) followed 1 hour later by 5-FU (1.5 g/m^2 iv bolus) and 24 hours later by oral folinic acid (15 mg/m^2 every 6 hours for 72 hours); and Day 14, doxorubicin (30 mg/m^2 iv) cycled every 28 days. We have now treated 12 patients with an identical regimen, except that forced alkaline diuresis was administered routinely and folinic acid was given at 50 mg/m^2 iv every 6 hours, continued until serum methotrexate levels were out of the toxic range. The 12 patients comprised seven males and five females, with a mean age of 61 years (range, 41–78). One patient had received previous chemotherapy. All patients had measurable disease, and only one had overt hepatic metastases. Performance status (Eastern Cooperative Oncology Group) was 1 in 11 patients and 2 in one. All patients had normal hematological indices prior to chemotherapy, a normal serum creatinine, and a creatinine clearance > 50 ml/minute. Two patients had elevation of serum bilirubin at 175 and 63 μmol/L, respectively, but

the remaining patients had normal liver function. The 12 patients received 35 courses of treatment. Following chemotherapy, two patients had partial response (> 50% reduction in tumor size), two had stable disease, seven had disease progression, and one was not evaluable due to death from septicemia. The major toxic effect was myelosuppression, with a wbc count nadir < 1000/mm^3 in four patients and 1000–2000/mm^3 in four patients. The two patients with abnormal liver function had nadir wbc counts of > 3000/mm^3. The median time of the wbc count nadir was Day 13. Three patients developed life-threatening septicemia during the neutropenic phase and another died of gram-negative septicemia. Thrombocytopenia was observed in only one patient, with a platelet count nadir of 54,000/mm^3. Mucositis was recorded in three patients, and an erythematous skin rash was recorded in two. Although all patients were given routine antiemetic treatment, four still experienced nausea and vomiting. There was no nephrotoxicity.

Clearly, it is premature to report the activity of this regimen in gastric cancer, since the confidence intervals on such a small patient sample are wide (0%–38%). However, the toxicity of the regimen deserves comment. In particular, we were alarmed by the high incidence of life-threatening infection, occurring in the context of neutropenia, which had not been anticipated on the basis of Klein et al's original report (1). The agents mainly responsible for the myelosuppression appear to have been methotrexate and 5-FU, which was reflected in the occurrence of the wbc count nadir on Day 13 of the treatment cycle. In terms of myelosuppression, doxorubicin was less important, and it was of interest that the patients with abnormal liver function did not develop significant neutropenia. The reason for the more severe myelosuppression in our study as compared to Klein's experience (1) is not clear, but it certainly cannot be attributed to the use of inadequate "folinic acid rescue." Moreover, the development of such myelosuppression suggests that there should be reservations regarding the routine outpatient use of this particular regimen, especially when the toxicity is compared to that with regimens such as FAM (5-FU, doxorubicin, and mitomycin) (2,3), and that its inclusion in adjuvant protocols is potentially hazardous.

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International Meeting on Gynecologic Oncology: Lymphatic Metastases in Gynecological Malignant Tumors

Rome, Italy, September 9–12, 1985

This meeting, which will focus on clinical significance, surgical techniques, and treatment of lymphatic metastases in gynecological malignant tumors, is scheduled to take place immediately before the XIth World Congress of Gynaecology and Obstetrics in Berlin, Germany (Sept 15–20).

In addition to the talks by invited speakers, a limited number of brief communications (in the form of poster) which are strictly related to the topics of the symposium will be accepted for presentation.

The official languages of the meeting are Italian and English; a simultaneous translation service will be available.

For further information, contact: Prof. Salvatore Mancuso, Department of Obstetrics and Gynaecology, University of Cagliari, Via Ospedale 46, 09100 Cagliari, Italy.

International Symposium on Labelled and Unlabelled Antibody in Cancer Diagnosis and Therapy

Baltimore, Maryland, September 12–13, 1985

This symposium, to be held at The Johns Hopkins Medical Institutions, will include sessions on monoclonal antibody in therapy, treatment of bone marrow with antibody for transplantation, radiolabeled antibody in diagnosis, dosimetry and physics of radiolabeled antibody, radiobiology of low-dose-rate radiation, therapeutic approaches with drug labels, radiolabeled antibody, and new diagnostic and therapeutic approaches.

For further information, contact: American College of Radiology, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3150).

Two Short Courses: Epidemiology and Toxicology

Houston, Texas, October 7–11, 1985

The New England Epidemiology Institute will present two consecutive short courses at the Shamrock Hilton Hotel in Houston. "Principles of Epidemiology" (Oct 7–9) will be taught by Drs. Kenneth Rothman and Stephen Lanes and Mr. Charles Poole. They will present modern concepts in epidemiology and their applications to the study of etiology, natural history of disease, and strategies in preventive medicine and public health. "Regulatory Toxicology" (Oct 10–11) will be taught by Dr. Edward Calabrese. He will discuss how information has been used and should be used in the process of setting standards for drinking water, ambient air, and the workplace.

No previous study of epidemiology, biostatistics, or toxicology is required for either course. Registrants may receive Continuing Medical Education Credits (AMA Category 1) and/or Certification Maintenance Credits from the American Board of Industrial Hygiene.

For further information, contact: New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Cincinnati Cancer Conference IV

Cincinnati, Ohio, November 1–2, 1985

The Bethesda Hospital, American Cancer Society, and University of Cincinnati College of Medicine are sponsoring this conference devoted primarily to: Ovarian Cancer, New Developments, and Melanoma.

The meetings will be held in the Hyatt Regency Cincinnati; the registration fee is $190. This continuing medical education activity meets the criteria for 11 credit hours in Category I of the Physician's Recognition Award of the AMA. The program is acceptable to the AAFP for 11 prescribed hours and to the ACOG for 11 cognate hours.

For further information, contact: Tom O'Connor, Course Manager, Bethesda Hospital, Inc, 619 Oak St, Cincinnati, OH 45206 (telephone: 513-569-6339).

Second International Symposium on Detection and Treatment of Minimal Residual Disease in Acute Leukemia

Rotterdam, The Netherlands, November 6–8, 1985

This symposium is focused on the detection and treatment of subclinical disease in acute leukemia, ie, the small numbers of neoplastic cells which frequently persist following successful remission induction therapy and cause relapse. The organizers are Drs. Anton Hagenbeek and Bob Löwenberg; topics will include new methods to detect residual leukemia, the value of new chemotherapy programs, and recent accomplishments in clinical bone marrow transplantation.

For further information and abstract forms, contact: J. W. van der Velden, MD, c/o Organizing Committee Leukemia Symposium, The Dr. Daniel den Hoed Cancer Center, PO Box 5201, 3008 AE Rotterdam, The Netherlands.

SOMPS First International Congress on Neo-Adjuvant Chemotherapy

Paris, France, November 7–9, 1985

Several international experts will participate in this congress, sponsored by the Service d'Oncologie Médicale Pitié-Salpétrière (SOMPS).

For further information, contact: SOMPS—First International Congress on Neo-Adjuvant Chemotherapy, Pavillon Jacquart, Hôpital de la Salpétrière, 47, boulevard de l'Hôpital, 75651 Paris Cedex 13, France (telephone: 570-28-29 or 570-28-30).

International Workshop on Quality of Life Assessment and Cancer Treatment

Milan, Italy, December 11–13, 1985

This workshop will be held at the Istituto Nazionale per lo Studio e la Cura dei Tumori, to acquaint oncologists interested in psychosocial oncology with the possibilities of implementing psychosocial and behavioral outcomes into cancer clinical trials. The program will be developed along two lines: (*a*) short didactic introduction into methodology and measurement in cancer clinical trials; and (*b*) application in trials.

For further information, contact: Scientific and Organizing Secretariat, Fondazione Floriani, Vicolo Fiori 2, 20121 Milan, Italy (telephone: 02-877.240/805.9070).

First International Symposium: Multimodality Treatment of Head and Neck Cancer

Genoa, Italy, December 17–18, 1985

The Istituto Nazionale per la Ricerca sul Cancro is organizing this conference as a forum for the most active and prominent investigators in cell kinetics, prognostic factors, diagnosis, staging, evaluation of response, and single-modality treatment of advanced stages of head and neck cancer. Particular emphasis will be given to combined modalities and second-line treatments.

For further information, contact: M. Merlano, MD, Istituto Nazionale per la Ricerca sul Cancro, Viale Benedetto XV, 16132 Genoa, Italy (telephone: 010-300770).

Counseling Strategies for Physicians

Oahu, Hawaii, March 24–29, 1986

The University of Texas at Tyler, in cooperation with The University of Texas Health Science Center at Houston, is sponsoring this meeting to introduce the latest techniques and research findings on matters that influence doctor-patient relationships. Faculty will discuss social change, family violence, addiction, and human sexuality as considerations in dealing with patients, and they will present problems likely to be encountered with specific groups of clients such as teenagers, minority groups, aging clients, and pregnant women.

The registration fee is $325; the program is approved for 17 hours in AMA/PRA Category 1 and is AAFP prescribed.

For further information, contact: Division of Continuing Education, The University of Texas Health Science Center at Houston, 1100 Holcombe, Rm 1501, PO Box 20367, Houston, TX 77225. Telephone—within Texas: 1-800-392-4900-737, Program Code 975 DLB; outside Texas: 1-800-332-8747.

Position Vacancy: Associate Director for International Affairs, National Cancer Institute

This position is located in the Office of International Affairs (OIA), Office of the Director, National Cancer Institute (NCI), Public Health Service, U.S. Department of Health and Human Services.

NCI interacts with scientists throughout the world to promote the advancement of cancer research, treatment, and prevention. These activities result in bilateral agreements with other countries for cooperative research, the collection and dissemination of scientific knowledge worldwide, and the increased use of computer technology to improve these information transfers. OIA is responsible for the planning, coordination, and management of these efforts within the NCI. OIA has an annual operating budget of approximately $9 million.

This is a Civil Service position in the Senior Executive Service (SES), with a salary range from $61,296 to $68,700 per annum. Candidates may be eligible for Physicians' Comparability Allowance up to $10,000 per year. (Alternately, candidates may be eligible for appointment in the Commissioned Corps of the U.S. Public Health Service.) The individual selected, if not presently in SES, must serve a 1-year probationary period.

Candidates must meet established mandatory professional/technical and managerial/executive requirements. Candidates will be further evaluated on the extent to which they exceed the minimum requirements and on additional factors including scientific reputation/peer recognition, training and education, and honors and awards. A copy of the requirements may be obtained by contacting Ms. Valerie Barbour, Personnel Management Branch, NCI, (301) 496-6864.

Applications (Personal Qualifications Statement SF-171) are to be sent to Ms. Barbour at: The National Cancer Institute, Bldg 31, Rm 3A-23, 9000 Rockville Pike, Bethesda, MD 20205. A current curriculum vitae and bibliography must accompany all applications. Applications from women and minority group members are especially solicited.

The National Institutes of Health is an EQUAL OPPORTUNITY EMPLOYER.

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